

THE AMERICAN JOURNAL OF PATHOLOGY

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Several investigators have noticed that treatment with folliculoid (or "estrogenic") estrane derivatives may cause squamous metaplasia and even cornification of the epithelium in the prostates, coagulating glands, ampullary glands and seminal vesicles of rodents. At the same time these substances induce marked proliferation of the connective tissue and smooth muscle tissue in the walls of these glands.¹⁻⁴ In the rat this metaplasia is much more difficult to produce than in the mouse and the epithelium of the seminal vesicles appears to be practically resistant to this effect except in the region of the duct.^{5,6}

Experiments on the mouse led Burrows⁷ and de Jongh, Querido and Stolte⁸ to conclude that the metaplasia normally produced by estrone can be prevented by simultaneous progesterone administration. In this connection it is perhaps worth mentioning also that according to Lipschütz, Vargas and Nunez⁹ certain tumor-inducing effects of folliculoids are likewise counteracted by progesterone and desoxycorticosterone.

In the present communication we describe experiments on rats indicating that, at least under certain conditions, a special type of epithelial metaplasia can be produced by the combined administration of estradiol with either progesterone or desoxycorticosterone acetate, while similar treatment with any one of these steroids alone causes no such change.

EXPERIMENTAL FINDINGS

In our first experimental group 60 castrate male albino rats weighing 40 to 65 gm. were divided into six groups of 10. One group served as controls and received only peanut oil injections; the others were treated with various hormones or hormone combinations. The daily dose was

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dissolved in 0.1 cc. of peanut oil and administered in two injections. All animals were castrated 2 days prior to the initiation of treatment. After 10 days of treatment the animals were killed and their sex organs weighed and examined histologically following fixation in Heidenhain's "Susa" mixture.

The upper portion of Table I summarizes the organ weights observed in the six groups of this experimental series. It will be seen that the weight of the seminal vesicles was increased by estradiol, but not significantly modified by progesterone or desoxycorticosterone acetate. In the combined administration of estradiol with progesterone or desoxycorticosterone acetate, the latter two compounds did not significantly influence the weight increase caused by estradiol. The weight of the prostate was slightly increased by progesterone and estradiol, but not by desoxycorticosterone acetate, and with combined treatment

TABLE I
Effect of Various Steroids On the Weight of the Accessory Sex Organs in Castrate Rats

Treatment	Dosage per day	Body weight in gm.		Seminal vesicle in mg.	Prostate in mg.	Preputial glands in mg.
		Initial	Final			
Oil		<i>55*</i> (45-65)	<i>102</i> (82-124)	<i>9.7</i> (7.5-12)	<i>29</i> (21-35)	<i>23</i> (15.5-28)
<i>a</i> -estradiol	400 γ	<i>53</i> (40-60)	<i>77</i> (70-94)	<i>36</i> (24-45.5)	<i>40</i> (30-54)	<i>26</i> (13-39)
Progesterone	4 mg.	<i>53</i> (45-60)	<i>90</i> (75-128)	<i>11.5</i> (9-16.5)	<i>42.5</i> (31-53)	<i>33</i> (20-56)
Desoxycorticosterone acetate	4 mg.	<i>54.5</i> (45-65)	<i>83</i> (75-97)	<i>10</i> (6-13.5)	<i>28.5</i> (23.5-34.5)	<i>23</i> (18-26)
<i>a</i> -estradiol and desoxycorticosterone acetate	400 γ	<i>53</i> (40-60)	<i>82</i> (67-89)	<i>38</i> (24-46.5)	<i>40</i> (20-48)	<i>29.5</i> (15-38)
	4 mg.					
<i>a</i> -estradiol and progesterone	400 γ 5 mg.	<i>55</i> (40-62)	<i>88</i> (81-94)	<i>34</i> (25-43)	<i>62</i> (49-84)	<i>35</i> (24-47)
<i>a</i> -estradiol	100 γ	<i>53</i> (45-60)	<i>74</i> (55-95)	<i>29</i> (25-33)	<i>38</i> (33-44)	<i>24</i> (19-31)
Progesterone	10 mg.	<i>48</i> (40-55)	<i>90</i> (85-100)	<i>12.5</i> (12-14)	<i>113</i> (95-130)	<i>36</i> (26-53)
Desoxycorticosterone acetate	10 mg.	<i>40</i> (39-42)	<i>72</i> (70-75)	<i>9</i> (7-10)	<i>24</i> (17-31)	<i>17</i> (10-20)
<i>a</i> -estradiol and desoxycorticosterone acetate	100 γ	<i>57</i> (50-70)	<i>75</i> (60-100)	<i>27</i> (24-35)	<i>38</i> (27-55)	<i>27</i> (19-36)
	10 mg.					
<i>a</i> -estradiol and progesterone	100 γ 10 mg.	<i>55</i> (45-65)	<i>81</i> (75-90)	<i>31</i> (29-38)	<i>116</i> (78-146)	<i>37</i> (23-52)

* In each instance the average weight is given in italics, and beneath it the range is indicated in parentheses.

with estradiol and progesterone or desoxycorticosterone acetate the actions of each of these compounds appeared merely to be summated without any indication of potentiation. The preputial glands were markedly stimulated by progesterone only and the effect was not noticeably influenced by simultaneous estradiol administration.

Although the effect of the above three steroids on the gross weight of the accessory sex organs was merely additive, histological examination of the seminal vesicles revealed a curious change in the quality of the response to estradiol in the group in which this compound was administered in combination with progesterone. The epithelium of the seminal vesicles, which was not influenced either by progesterone or by estradiol alone, exhibited signs of marked proliferation and stratification in the group treated with both these compounds. Combined treatment with estradiol and desoxycorticosterone acetate did not elicit a similar epithelial proliferation in this experimental series.

It is well known that desoxycorticosterone acetate and progesterone are chemically and pharmacologically closely related compounds. Chemically the former differs from the latter only in the presence of an acetoxy- group in position C₂₁, and pharmacologically both substances possess luteoid and corticoid activity. It was therefore decided to repeat these experiments under otherwise identical conditions but giving a smaller dose of estradiol in combination with a larger dose of progesterone and desoxycorticosterone acetate. It was hoped that under such conditions the similarity in the effect of the latter two compounds would become evident and the curious type of epithelial metaplasia more readily demonstrable. The average weights of the accessory sex organs, as well as other details of this experiment, are summarized in the last five horizontal sections of Table I. It will be seen that even at the very high dosage level of 10 mg. per day, neither desoxycorticosterone acetate nor progesterone caused any significant change in the weight of the seminal vesicles, but while desoxycorticosterone acetate was also without effect on the weight of the prostates and preputial glands, progesterone definitely stimulated the latter two organs. In this, as in the first experimental series, the effect of the three steroids on the gross weight of the accessory sex organs was merely additive.

Histologically the lining of the seminal vesicles in the groups treated with progesterone and desoxycorticosterone acetate consisted of a single layer of epithelial cells which were perhaps slightly larger than those of the controls treated with oil, but never showed any sign of stratification. Similarly, none of the animals treated with estradiol alone showed stratification of the epithelium in the seminal vesicles, although they did exhibit the usual fibromuscular proliferation. On the other hand,

in the two groups receiving estradiol in combination with progesterone or desoxycorticosterone acetate, every animal exhibited marked proliferation and stratification of the epithelium and, although this never led to infiltration through the basement membrane into the muscular wall, it did, in many instances, completely obliterate the lumen of these glands. Histologically *we are not dealing with a squamous metaplasia, but merely with a stratification of the epithelium*, the cells of which retain their rather irregular cuboidal or polygonal shape. In certain instances we noted a good deal of sloughing of surface cells, which was reminiscent of holocrine secretion. Cornification was never observed. (Figs. 1 to 6.)

DISCUSSION

Our observations indicate that even compounds such as progesterone and desoxycorticosterone acetate, which have no obvious effect on the gross weight of the seminal vesicles in the castrate male rat, possess the latent potentiality of influencing these structures. This fact can be made obvious by administering these compounds in conjunction with estradiol. We were surprised to note, because of the previously mentioned experiments of Burrows⁷ and de Jongh, Querido and Stolte,⁸ who prevented estrone from causing squamous metaplasia and keratinization by giving it in combination with progesterone, that combined treatment with progesterone or desoxycorticosterone acetate causes this type of epithelial metaplasia. It must be emphasized, however, that, unlike these investigators, we experimented on the rat and that the histological type of the metaplasia produced by us differs significantly from that seen by the earlier workers. In these short-term experiments combined treatment with progesterone or desoxycorticosterone acetate and estradiol never caused tumor formation, but it will remain for further experiments to show what the ultimate fate of this abnormal epithelial growth will be if treatment is continued over a long period.

We omitted a detailed description of the histological appearance of the prostates and preputial glands since they showed no noteworthy change except that progesterone stimulated their epithelium, an effect which was not modified by estradiol. Metaplasia was never observed in these organs. In contradiction to Hooker and Collins,¹⁰ and in confirmation of the work of Paschkis¹¹ and Clausen,¹² we can say that desoxycorticosterone acetate is not a testoid compound; that is, it does not stimulate the accessory sex organs of the castrate male when given by itself. Greene, Burrill and Thomson¹³ and Clausen¹² claimed that progesterone is definitely testoid as judged by its effects on the accessory sex organs of castrate males, but our experiments reveal that it

possesses only a stimulating action upon the prostate and preputial glands and fails to increase the weight of the seminal vesicles.

SUMMARY

Experiments on immature castrate albino rats indicate that treatment during 10 days with estradiol, progesterone, or desoxycorticosterone acetate alone causes no significant change in the epithelium of the seminal vesicles, but if the same dose of estradiol is given during the same time in combination with either progesterone or desoxycorticosterone acetate, marked proliferation and stratification of the epithelium of the seminal vesicles is readily and consistently induced.

As an incidental finding it is noted that in immature castrate rats desoxycorticosterone acetate in doses up to 10 mg. per day fails to increase the weight of the seminal vesicles, prostates, or preputial glands. Progesterone enlarges the prostates and preputial glands under similar conditions, but fails to increase the size of the seminal vesicles even at this high level of dosage.

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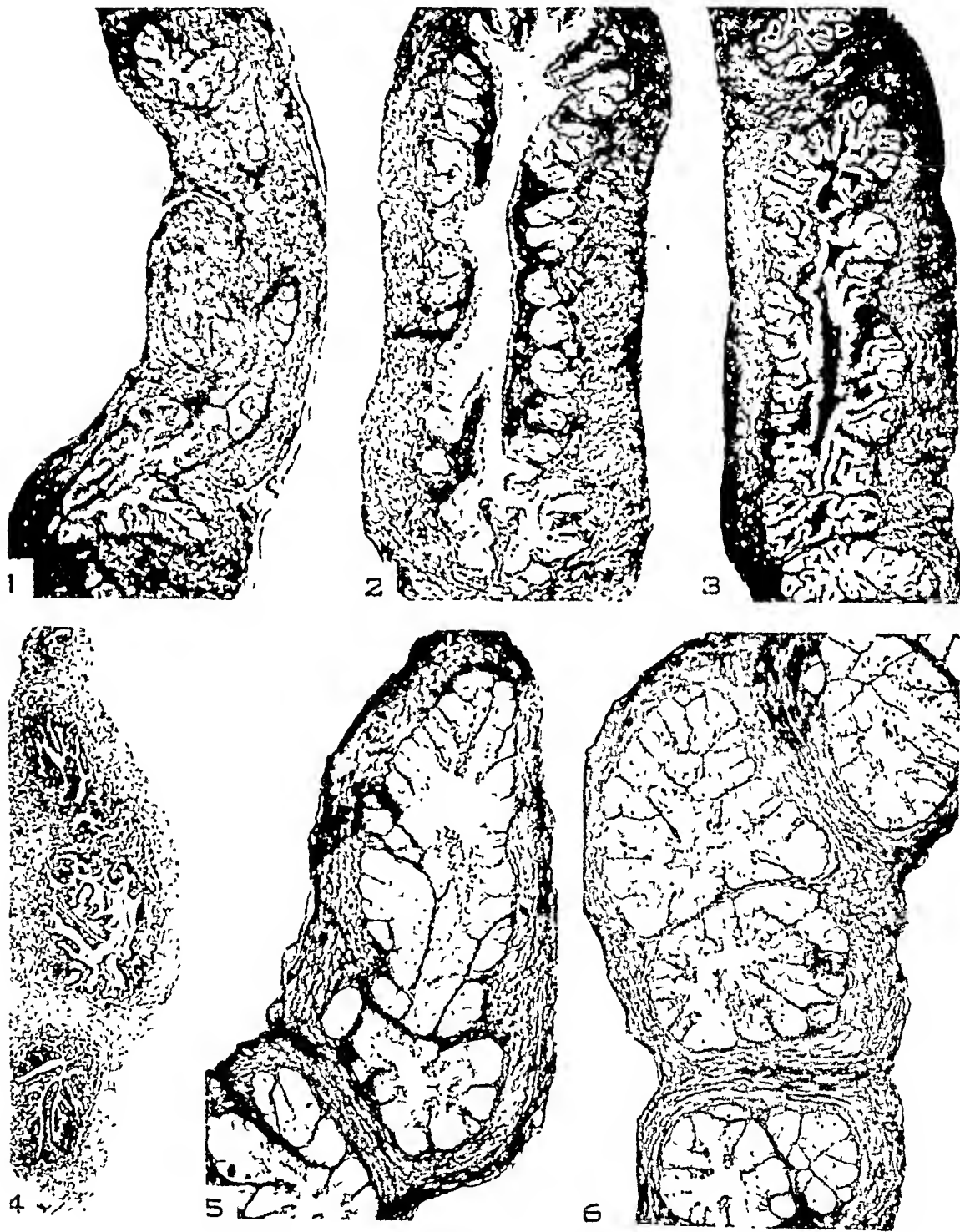
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DESCRIPTION OF PLATE

PLATE I

- FIG. 1. Seminal vesicle of castrate control animal treated with oil, showing poor development of epithelium and of fibromuscular coat.
- FIG. 2. Seminal vesicle of castrate rat treated with estradiol, showing proliferation of fibrous tissue and musculature without any significant change in the epithelium.
- FIG. 3. Seminal vesicle of castrate rat treated with progesterone. The epithelium is slightly higher than in the control but forms a single layer only.
- FIG. 4. Seminal vesicle of castrate rat treated with desoxycorticosterone acetate. The epithelium is slightly higher than in the control but forms a single layer only.
- FIG. 5. Seminal vesicle of castrate rat treated with estradiol and desoxycorticosterone acetate, showing marked proliferation and stratification of the entire epithelial surface.
- FIG. 6. Seminal vesicle of castrate rat treated with estradiol and progesterone, showing stratification of epithelium similar to that seen in Figure 5.





THE PRODUCTION OF CIRRHOSIS IN THE LIVER OF THE NORMAL DOG BY PROLONGED FEEDING OF A HIGH-FAT DIET *

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The first experimental indication that the long-continued presence of large amounts of fat in the liver gives rise to fibrosis was obtained in the completely depancreatized dog kept alive with insulin.¹ When maintained on a diet of lean meat, sucrose, salts and vitamin supplements, depancreatized dogs develop fatty livers.² Although as much as 32 per cent of fatty acids may appear in the liver as early as 3.5 weeks after excision of the pancreas, the rate of infiltration of fat in the liver is highly variable.³ It required about 16 weeks for all livers to attain a fatty-acid content in excess of 15 per cent. Once attained, however, fatty livers remain in such animals for long periods, and in one dog such a liver was observed as late as 3 years after pancreatectomy. But if the animals survived long enough, a spontaneous decline in the fat content of the liver might occur. In three dogs that survived from 4.2 to 5.5 years, nearly normal percentages of fatty acids were found, although the total amount of fatty acids present was still in excess of the normal because the size of the liver failed to regress as the fat left it.

The earliest evidence of hepatic fibrosis in a depancreatized dog was observed at an interval of 1.5 years after pancreatectomy.¹ Hepatic fibrosis developed in 8 of 16 dogs that had been kept alive from 2.6 to 5.5 years. In 4 of these, well advanced portal cirrhosis of the liver was found. By the time the most severe type of cirrhosis appeared, the fat content of the liver had been reduced to normal, and these livers showed little or no evidence that a marked infiltration had preceded the fibrosis.

The present report demonstrates that fibrosis and cirrhosis of the liver develop in *normal* dogs when their livers are kept excessively fatty by the continued administration of a high-fat diet.⁴ This new observation involving fatty livers of dietary origin, together with that previously made on the depancreatized dog¹—that the fatty liver occurred even though the diet fed was relatively low in fat—establishes a high

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‡ Deceased.

fat content in the liver as an important causative agent in the production of hepatic cirrhosis.

EXPERIMENTAL FINDINGS

The dogs recorded in this study were fed twice daily, at 8:00 a.m. and at 4:00 p.m., a mixture of lean meat, lard and bone ash. Each dog received daily 7 gm. of lean meat and 10 gm. of lard per Kg. of its initial body weight, and 1 to 3 gm. of bone ash with each meal. Vitamins A and D were supplied as cod-liver oil, and the vitamin B complex as Galen B (Vitab, type II, liquid *). One gm. of Cowgill's salt mixture⁵ was added to the evening meal.

Considerable variation was observed in the appetites of the dogs for this high-fat diet. All food not voluntarily eaten was force-fed. The dietary intake of each dog is recorded below:

Dog F-3. Ate well throughout experiment. Vomited only four times.

Dog F-6. Ate well for first 89 weeks, without vomiting. For the next month it was force-fed but vomited frequently. It then regained its appetite and continued to eat well, with only infrequent vomiting, until the 159th week. For the remainder of its period of survival it had to be force-fed every other meal, but vomited infrequently.

Dog F-20. Was force-fed throughout the period of observation. No vomiting occurred for the first 5 months, but for the next 2 months it vomited after almost every meal. No vomiting was then observed until the last 8 days of survival.

Dog F-28. Survived for 155 weeks. With the exception of 7 weeks it received the high-fat diet during the entire period of observation. The feeding of the high-fat diet was interrupted on two occasions, at 8 months and 15 months after the start of the experiment. During these two interruptions (7 weeks in all) it received a lean-meat diet supplemented with bone ash, Cowgill's salt mixture,⁵ cod-liver oil and Galen B. Force-feeding of the high-fat diet was resorted to almost entirely. Vomiting occurred only infrequently.

Dog F-29. Force-fed throughout; no vomiting.

Dog F-33. Ate well for first 2 months. Force-feeding was necessary thereafter, except for occasional meals. Vomited infrequently.

Dog F-39. Ate well for first 2.5 months. Force-fed thereafter. Vomited infrequently.

Dog F-40. Ate well for first 15 days. Force-fed every meal thereafter.

Dog F-41. Ate well for first 2 months. Thereafter was force-fed the high-fat diet except for short intervals of 3 and 2 weeks, during which

* This concentrate was generously supplied by the Vitab Corporation, Emeryville, Calif.

time fat was excluded. No vomiting occurred until the last 18 days, when this became frequent.

Dog F-43. Force-fed throughout. No vomiting.

Dog F-44. Ate well for first 2 months. Force-fed thereafter. Vomited only during last 3 days. Fat removed from diet for 1 month, *i.e.*, between 4th and 5th month.

Dog F-45. Force-fed throughout. No vomiting.

Dog F-46. Force-fed throughout. Vomited infrequently during last month.

Dog F-48. Ate well for first 6 months. Force-fed thereafter. Vomited every third meal during last 10 days of its survival.

Dog F-49. Ate well during first 10 months. Force-fed every meal thereafter. Vomited occasionally during first 8 months; not at all thereafter.

Dog F-50. Ate well for first 8 months. Force-fed thereafter. Vomited frequently during entire period of observation.

Dog F-59. Ate well for first 2 months. Force-fed thereafter. No vomiting for first 5 months, but vomited frequently during remainder of period.

The following stains were used for microscopic examination of the livers: hematoxylin and eosin; aniline blue for connective tissue; phosphotungstic acid and hematoxylin for connective tissue; hematoxylin and van Gieson's for connective tissue; sharlach R for fat. Hematoxylin and van Gieson staining for connective tissue was found most satisfactory for black and white photographic reproduction.

The method employed for the determination of fatty acids has been described elsewhere.⁶ A mixed sample of the whole liver was used for this analysis.

RESULTS

The pathologic changes observed in the livers of the 17 dogs recorded in Table I have been grouped according to the progress of the lesion. It should be recognized, however, that very sharp distinctions cannot be made because of imperceptible transitions from one phase to another. In group I have been recorded the livers showing the earliest changes; this consisted of fatty infiltration of the liver cells. Group II includes the livers that showed an increased prominence of the intra-lobular or intercellular connective tissue, as well as those in which an actual fibroblastic proliferation and diffuse cirrhosis were observed. Livers showing nodular cirrhosis have been placed in group III; this resulted from a regeneration of liver cells that had been divorced from their normal portal and central relationship by the fibrosis. Five of the livers fall into group I, 10 into group II and 2 into group III.

TABLE I
Summary of Dogs

Dog no.	Sex	Duration of experiment	Died (D) or sacrificed (S)	Weight of dog			Liver			Group classification
				Initial (Kg.)	Maximum (Kg.)	Final (Kg.)	Weight (gms.)	Total fatty acids (per cent)	Gross and histologic appearance	
F-3	F	5 wks.	S	7.0	7.2	7.1	325	30.0	Marked fatty infiltration; no fibrosis; very little glycogen	I
F-6	F	3 yrs. + 26 wks.	S	5.6	7.9	4.5	...	5.4	Very nodular liver; cirrhosis with hyperplasia	III
F-20	F	36 wks.	D	12.1	15.5	13.7	485	12.4	Pale fatty liver; good cirrhosis with bile retention	II
F-28	M	2 yrs. + 51 wks.	S	7.6	9.0	8.0	231	20.9	Fatty liver; early slight fibrosis	II
F-29	F	15 wks.	D	8.2	8.2	7.6	943	35.4	Fatty green liver; early fibrosis and bile retention	II
F-33	F	37 wks.	D	6.6	7.5	7.5	475	25.4	Fatty, slightly green liver containing excess fibrous tissue	II
F-39	M	32 wks.	D	9.7	11.4	11.2	467	14.0	Fatty liver with well advanced cirrhosis throughout	II
F-40	M	10 wks.	D	6.1	6.5	6.1	420	25.7	Fatty liver with marked bile retention; no fibrosis	I
F-41	F	1 yr. + 3 wks.	D	11.2	16.9	12.8	695	20.2	Liver green, fatty, firm and granular, showing advanced cirrhosis	II
F-43	F	24 wks.	D	6.6	8.6	7.1	448	27.4	Fatty liver without fibrosis	I
F-44	F	42 wks.	D	7.0	8.6	8.0	423	16.4	Fatty liver with diffuse fibrosis	II
F-45	F	13 wks.	S	9.1	12.2	12.1	443	37.6	Fatty liver; no fibrosis	I
F-46	M	13 wks.	S	14.1	16.8	16.8	426	23.6	Fatty liver without fibrosis	I
F-48	F	1 yr. + 3 wks.	D	10.1	15.0	10.7	1600	17.0	Fatty green liver; advanced cirrhosis	II
F-49	M	1 yr. + 7 wks.	S	11.4	15.1	10.5	720	13.6	Fatty green liver; cirrhosis with adenomatous regeneration	III
F-50	F	17 wks.	D	7.7	8.8	7.4	1026	46.6	Fatty liver with bile retention; early, diffuse fibrosis	II
F-59	M	23 wks.	S	10.0	12.9	10.0	1079	37.4	Fatty green liver; early, diffuse fibrosis	II

Early Fatty Changes in the Liver
(See Fig. 1)

The liver of dog F-46 is representative of the accumulation of abnormal amounts of fat within the liver cells at a stage in which no increase in connective tissue has yet occurred. This animal was sacrificed after it had been maintained for 3.2 months on the high-fat diet. Its liver was not unusual in gross appearance except for its fattiness. It contained 23.6 per cent fatty acids.

Microscopically, the liver showed a peculiar type of fatty infiltration similar to that noted in other animals of this series. Fat was present in all liver cells, generally in fine vacuoles, so that it more nearly resembled glycogen storage. Some cells, however, contained large fat globules. The cells distended with vacuoles tended to be larger than if they had been filled with glycogen. With a fat stain (sharlach R) the livers of this group showed fat in very large amounts in practically every hepatic cell. The cell membranes were generally more conspicuous than normal, each cell being separated by a rather wide, deeply staining, limiting membrane. There was no visible increase in connective tissue.

Variations. In an animal sacrificed after only 35 days (F-3), the liver contained 30 per cent of fatty acids. The fat had a midzonal distribution although many whole lobules were affected. In an animal sacrificed after 5 months and 18 days (F-43), a hyaline atrophy of the cells was observed around the portal spaces. These particular cells contained neither fat nor glycogen, although elsewhere in the lobule practically every cell was filled with fat.

Fibrous Changes in the Liver
(See Figs. 2, 3 and 4)

The pathologic changes in the livers of this group ranged from a slight but definite proliferation of young connective tissue (which extended out from portal areas) to an extensive diffuse fibrosis that appeared to follow no particular arrangement or pattern. The earliest changes occurred in relation to the portal spaces. In more advanced cases thin strands of connective tissue surrounded many of the lobules and also extended *into* the lobules to the vicinity of the central veins. This fibrosis proceeded without regard to portal or central relationship, so that the fully developed picture was that of an aimless proliferation of fibrous connective tissue involving the entire liver and ignoring its normal architecture. The following description is representative of the group.

Dog F-48. This animal died after it had been maintained on the

high-fat diet for 1 year and 3 weeks. The liver was greenish yellow in color and smooth on the surface. The cut surface was mottled with bile, and the tissue throughout seemed firmer than usual. *Microscopically*, the liver showed considerable fat and a great deal of bile, the bile having collected in the interstitial tissue and in many cells. The marked proliferation of connective tissue, which was found throughout the liver, bore no relation to the portal areas or to the lobules. The lobular arrangement had entirely disappeared. The connective tissue had grown in no definite or characteristic manner but was found everywhere infiltrated between cells, sometimes in large amounts, sometimes in thin strands. This actively growing connective tissue had incorporated many degenerating liver cells as it infiltrated the normal tissue.

Nodular Cirrhosis of the Liver
(Figs. 5, 6 and 7)

The two livers of group III (F-6 and F-49) differed from those of the preceding group in having present a distinct-nodular or adenomatoid hyperplasia of islands of liver cells. The fibrosis was general, as in the livers of group II. But the diffuse and aimless distribution of the connective tissue had so changed that now slender strands or thick bands of fibrous tissue completely surrounded masses of liver cells. These masses of liver cells were lacking in their usual portal or central connections. The nodules were of all sizes and were scattered throughout the liver. The following description is that of the liver of dog F-6. This animal was sacrificed after it was kept on the high-fat diet for about 42 months.

The gross appearance of the liver was striking. It had a yellowish tawny color and was extremely irregular in shape. Its borders were sharp except at the lower edge of the right lobe, where, because of the nodules present, the border was rounded. The surface was nodular, the nodules ranging in size from a few millimeters to 2 cm. in diameter. These projected above the surface of the liver as much as 1 cm. Some were practically extruded and connected with the liver by a bit of capsular material. The four lobes consisted entirely of adenomatous masses of liver tissue. On first impression it suggested a carcinoma of the hepatoma type, but on section it looked like a remarkable adenomatoid hyperplasia in a cirrhotic liver.

Microscopic Examination. Most of the liver cells appeared relatively normal, except that they were not aligned in cords and had lost their relation with central veins and portal areas. They resembled post-mortem liver cells in that they contained little or no glycogen, this presumably having been hydrolyzed. The cells were grouped into

rounded masses by either thick or thin strands of connective tissue that gave the liver the adenomatous picture described above. These areas varied in size from microscopic to very large nodules observed grossly. The cells within adenomatous areas contained considerable fat in large or small vacuoles and were similar to the cells in the smaller nonnodular patches of diffuse fibrosis. In some nodules there had been a diffuse proliferation of connective tissue; as noted above, these did not have the normal liver structure and seemed obviously to be regenerated hyperplastic liver tissue. Some cells contained two nuclei, but mitotic figures were not seen. Bile was present in large inspissated clumps, distended canaliculi and filled some of the smaller bile ducts. In the connective tissue radiating from portal areas there were many newly formed bile ducts, some with several layers of epithelium. Many such ductlike structures had no lumina. The condition was a remarkably good example of diffuse cirrhosis of the liver with bile-duct proliferation, bile retention and adenomatoid regeneration.

DISCUSSION

The results of this investigation demonstrate the occurrence of a diffuse cirrhosis of the liver in dogs maintained on a high-fat diet. The first deviation from the normal was the accumulation of fat within the liver cells. This ranged from small, fine droplets to large globules which distended and distorted the liver cell. Practically every liver cell was affected. The cell boundaries became more distinct so that the cells were sharply outlined. This was accompanied by a hyaline atrophy or coagulative degeneration affecting cells adjacent to the portal veins. This picture was supplemented by the appearance of connective tissue fibers which first extended out from the portal spaces. Later there appeared a diffuse proliferation of connective tissue without regard to the normal architecture of the liver. In several cases the fibrosis was accompanied by bile-duct proliferation. In cases of longer duration, more or less distinct circumscribed nodules of regenerating liver cells were seen, while in two animals that survived more than 1 year a cirrhosis with discrete gross and microscopic nodules of hyperplastic liver tissue was observed.

It would seem that the diffuse fibrosis and cirrhosis observed in these dogs was produced by a low-grade injury maintained over a long period of time. That the injury to the liver was of mild degree was evident by the very slight or almost complete absence of inflammatory reaction. Neither inflammation nor infection appeared to be involved here, for no pathologic findings other than the liver fibrosis were observed in these dogs at autopsy. The coagulative degeneration of some of the liver

cells no doubt aided in stimulating fibrosis. Another factor to be considered here is interference in the hepatic circulation, since congestion was noted in several of the livers examined. Whether or not large amounts of neutral fat within the hepatic cell act as a *direct stimulus* to fibroblastic proliferation cannot be determined by the observations recorded here.

SUMMARY

1. Diffuse cirrhosis of the liver was produced in the *normal* dog by the long-continued administration of a high-fat diet.

2. The hepatic lesions produced varied from early, slight or diffuse fibrosis to very nodular cirrhosis in which adenomatous regeneration had occurred.

3. The results of the present investigation point to continued fatty infiltration as an important causative factor in the production of liver cirrhosis.

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DESCRIPTION OF PLATES

PLATE 2

FIG. 1. Dog F-29. Peculiar type of fatty infiltration in large globules and in fine, small vacuoles resembling glycogen storage. This liver contained 35.4 per cent fatty acids. Hematoxylin and van Gieson stain. $\times 530$.

FIG. 2. Dog F-59. Very fatty liver with early diffuse increase in connective tissue. This liver contained 37.4 per cent fatty acids. Hematoxylin and van Gieson stain. $\times 530$.

FIG. 3. Dog F-44. Diffuse fibrosis disrupting the usual lobulated appearance of the liver. This liver contained 16.4 per cent fatty acids. Hematoxylin and van Gieson stain. $\times 130$.

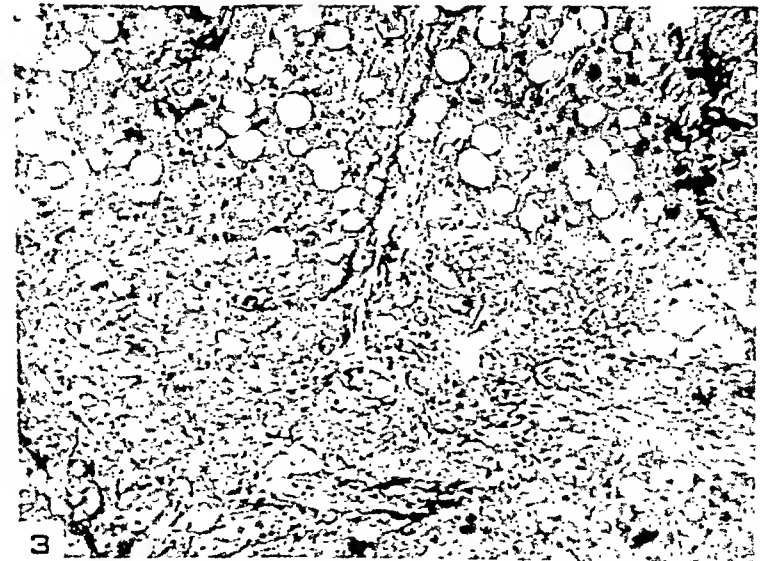
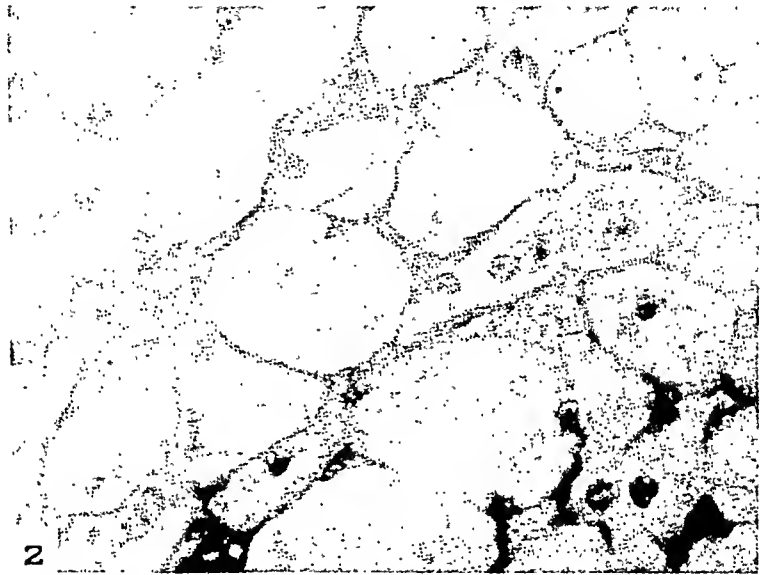
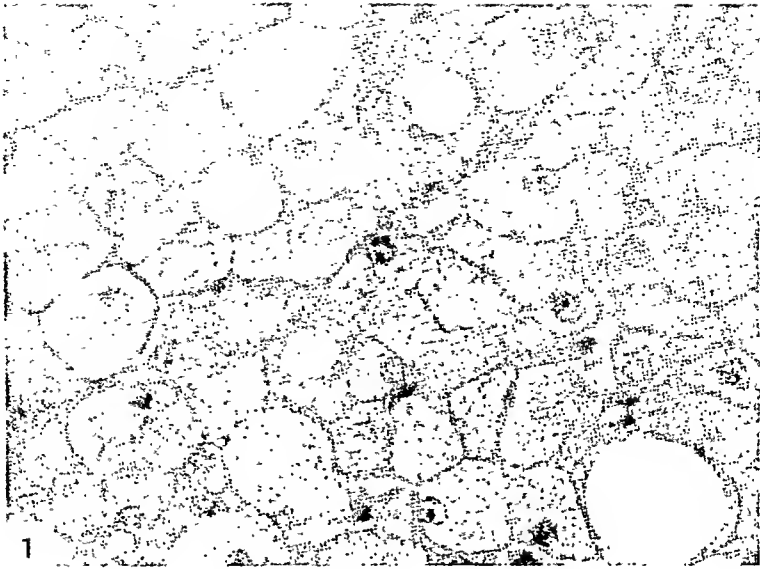
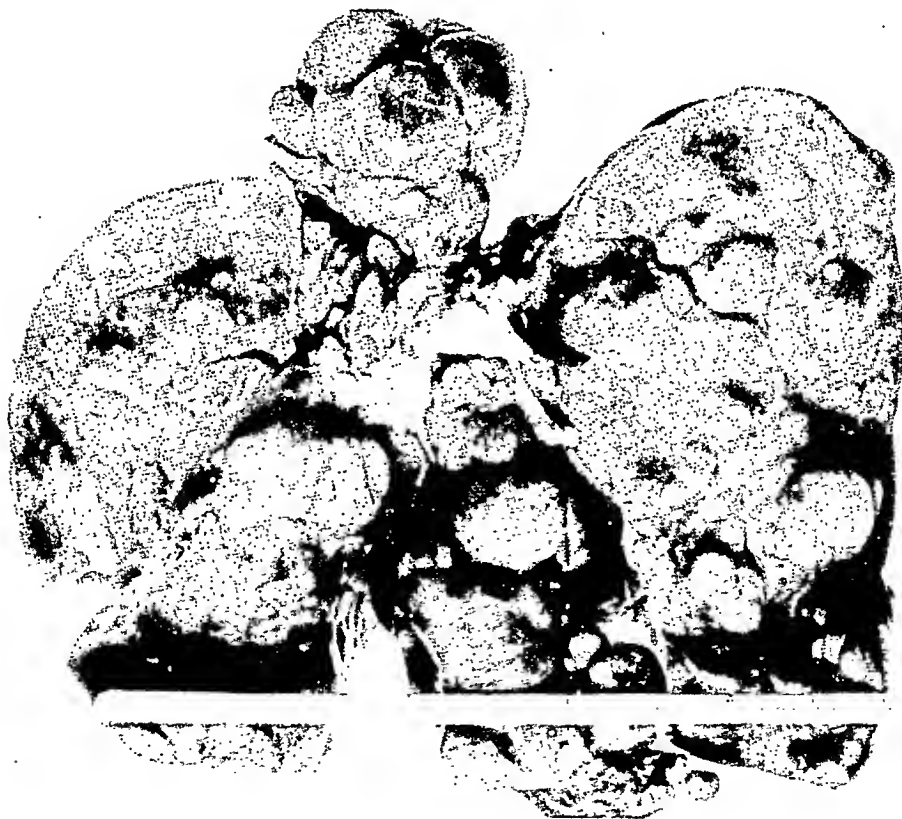
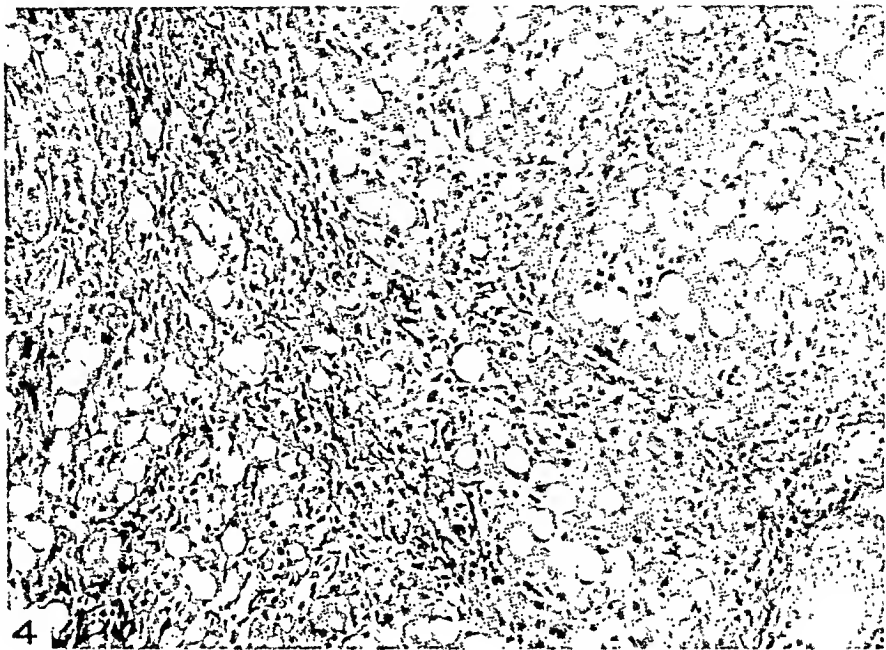


PLATE 3

FIG. 4. Dog F-39. A good example of the pure fat type of cirrhosis. There is a great deal of relatively young and actively proliferating connective tissue. This liver contained 14 per cent fatty acids. Hematoxylin and van Gieson stain. $\times 150$.

FIG. 5. Dog F-6. Cirrhosis of the liver with adenomatoid hyperplasia.



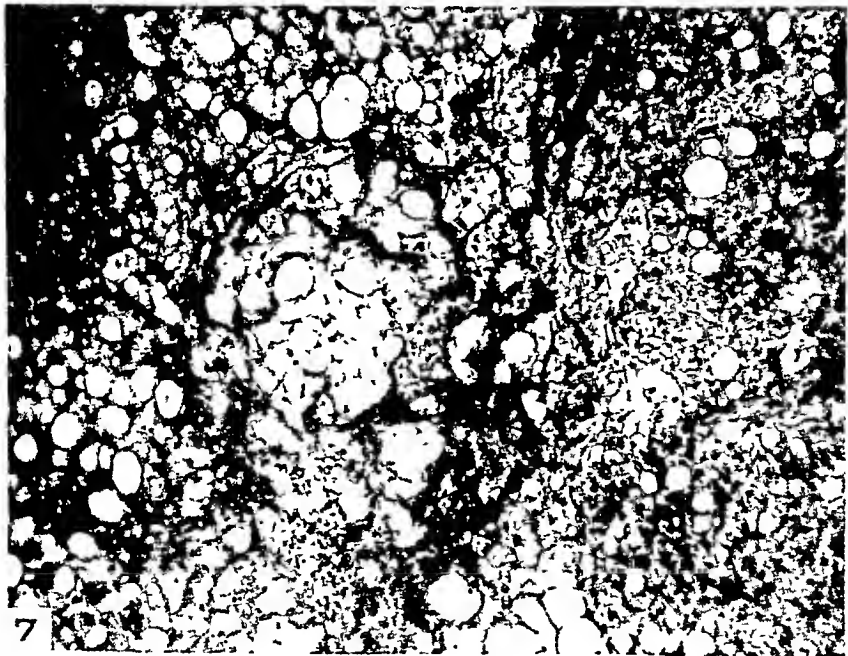
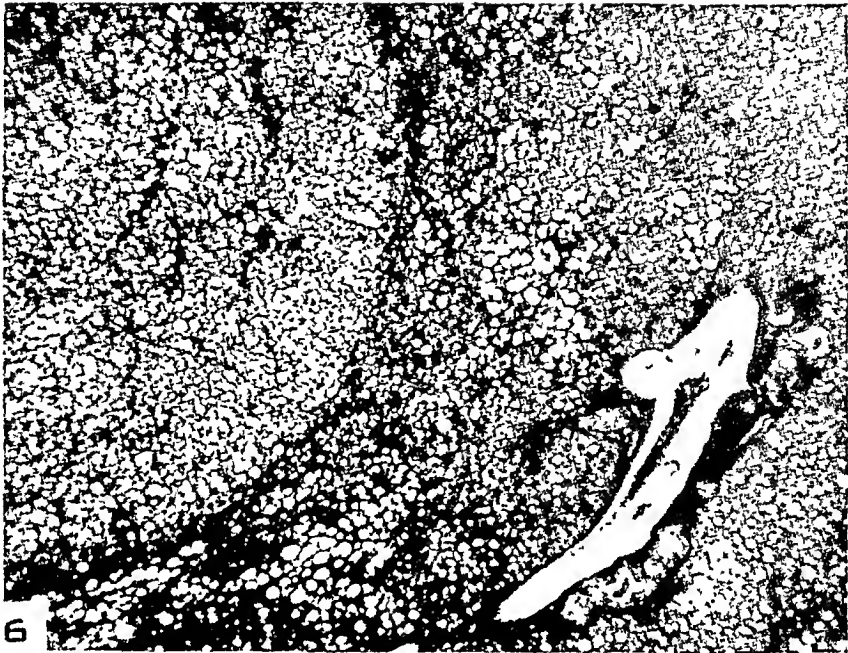
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Production of Cirrhosis by High-Fat Diet

PLATE 4

FIG. 6. Dog F-49. Advanced cirrhosis of the liver with a circumscribed nodule isolated from the portal vein. This liver contained 13.6 per cent fatty acids. Hematoxylin and van Gieson stain. $\times 40$.

FIG. 7. Connective tissue proliferation in the liver of dog F-49 showing an incipient nodule not fully encapsulated. Hematoxylin and van Gieson stain. $\times 150$.



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Production of Cirrhosis by High-Fat Diet



PATHOLOGY OF STAPHYLOCOCCAL PNEUMONIA COMPLICATING CLINICAL INFLUENZA *

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From the latter part of December, 1940, through most of January, 1941, there occurred an outbreak of acute respiratory infections in and around Boston. Some of the epidemiological, clinical and laboratory aspects of this epidemic were reported by Pearson, Eppinger, Dingle and Enders.¹ These authors succeeded in isolating influenza A virus from typical uncomplicated cases. They also presented serological evidence that a large number of patients with pneumonia who were admitted to the Boston City Hospital during this time had had contact with influenza A virus, suggesting a possible relation between the two diseases. Among the patients with pneumonia who were seen during this period, there was an unusually large number in whom *Staphylococcus aureus* was the only, or the predominant, organism found in cultures from sputum, lungs, blood, or pleural fluid. A brief account of some of the latter cases has already been given.² We wish here to present the pathological features of the staphylococcal pneumonias observed during this influenza outbreak.

The association of the staphylococcus with certain cases of bronchopneumonia has been recognized since the influenza pandemic of 1889. During that epidemic and even earlier, necrosis of the bronchi and alveolar structure of the lung occurring in cases of influenzal pneumonia had been noted by a number of writers and staphylococci had been cultured from some cases.³ Netter,⁴ in a study of the bacteriology of bronchopneumonia, found staphylococci in 3 of 39 cases in adults from whom a single organism was obtained in pure culture and in 8 of 14 additional patients with mixed infections. Two of those with pure cultures of staphylococcus had multiple disseminated foci (abscesses?) and in the third there was "splenization" of the lungs. In infants and children he found staphylococci in 5 of 25 cases that yielded pure cultures and in 8 of 17 patients with multiple organisms. For the pneumonias complicating "la grippe" in 1889-90, he stated that there was unanimous agreement that the organisms were similar to those of other bronchopneumonias. He found staphylococci in 2 of 8 such cases, 1 associated with pneumococcus and the other with a streptococcus. Fraenkel⁵ noted multiple abscesses in the influenzal bronchopneu-

* Received for publication, April 2, 1942.

monias that occurred in 1889 and described them as characteristic of the pathology of staphylococcal infection in the lungs.

During the pandemic of 1918, the staphylococcus was not an important causative organism in any significant portion of the complicating pneumonias that were studied carefully by many different observers. There were some exceptions, notable among which were the cases reported by Chickering and Park⁶ from Camp Jackson. In this camp the staphylococcus played the predominant rôle in the severe pneumonias observed during the influenza epidemic. Among 1409 patients with pulmonary complications, there were 385 deaths. Cultures, made from the lungs of 312 of the fatal cases, yielded a staphylococcus as the only or predominant organism in 153 instances. The disease in these cases was often fulminating, but if it was of long enough duration it was characterized by innumerable abscesses in the lung. In the patients who died earlier in the disease microscopical sections of the lungs showed intense congestion with rupture of alveolar walls and exudation of red cells and serum into the alveoli.

The report from no. 3 Canadian General Hospital⁷ indicated that there the staphylococcus was next in importance only to the influenza bacillus. A study of 86 fatal cases of pneumonia in this hospital revealed all the various pathological findings described as characteristic of the pulmonary complications of influenza: bronchitis; peribronchial abscesses, scattered or clustered; bronchopneumonia, nodular or interstitial, with hemorrhage, congestion and edema; bronchiectasis, especially in the cases of longer duration; lobular pneumonia with abscesses; diffuse congestion and edema in the rapidly fatal cases; areas of collapse; hemorrhagic infarcts; emphysema with bullae; non-purulent effusions in many instances and thin or thick pus in others; subpleural hemorrhages in most cases, and diffuse congestion of the trachea (but not the larynx). Bacteriological studies were done in 67 of these cases. *Staph.* (mostly *aureus*) was found as the only or predominant organism in 14 (21 per cent) of the lungs, as compared with 30 cases (45 per cent) in which influenza bacilli were obtained. Altogether, the staphylococcus was found in 51 cases (76 per cent) while influenza bacilli were identified in 90 per cent of the cases from one source or another. Cultures of the heart's blood were done in 50 of these cases and 25 were positive. *Staph. aureus* was obtained in 8 cases, the influenza bacillus in 2 and pneumococci or streptococci in the others. It is of interest that at the no. 5 General Hospital and no. 25 Stationary Hospital the staphylococcus was obtained from only 1 of 44 blood cultures and was not recovered from any of the cultures of the lungs or bronchi in 46 similar cases.⁸

Staph. aureus also played an important part in the complications

of influenza noted in the military hospital at Malta.⁹ Among 50 cases, this organism was predominant in the sputum of 7 out of 9 fatal cases and in 9 patients who recovered from severe infections. In 9 of 11 autopsies there was evidence of infection with staphylococcus. All had extensive congestion and edema, but bronchopneumonia was not always widespread. In the involved areas the exudate was scanty, but there was a tendency to abscess formation. Empyema was found in 3 cases, all of which yielded *Staph. aureus*.

In a study by Winternitz, Wason and McNamara¹⁰ of 82 autopsies in cases of influenza at New Haven, the staphylococcus was found alone in the lungs in only 1 case. It was recovered with other organisms in 19 cases, including 4 of the 34 acute cases, 9 of the 36 with necrotizing, and 6 of the 12 with organizing, pneumonia. Winternitz and his co-workers, in commenting on Chickering and Park's⁶ observations, noted that necrotization and abscess formation were striking features of the pathology of the epidemic even when the staphylococcus was not found. They could demonstrate no relation between the bacteriological findings and the distribution or the type of the pneumonic process, and that was also the experience of the no. 3 Canadian General Hospital.⁷

The pathological picture in the lungs which was so consistently observed in the 1918 pandemic has also been thoroughly described by numerous other workers, notably MacCallum,¹¹ Goodpasture and Burnett,¹² Goodpasture¹³ and Wolbach.¹⁴ All of the latter writers, as well as Winternitz, Wason and McNamara,¹⁰ described a hyaline membrane in the dilated bronchioles, in the alveolar ducts and sometimes in the subtended alveoli. There was always an associated necrotizing process of the tissue with an acute exudative reaction, hemorrhage, edema and necrosis of alveolar walls. Goodpasture¹³ found the hyaline membrane in 70 per cent of cases examined at the height of the epidemic. In cases of secondary infection, however, the membrane was replaced by an extensive necrosis of the pulmonary tissue. Later, however, Brannan and Goodpasture¹⁵ presented evidence to indicate that the hyaline membrane was not pathognomonic of influenza but probably represented a nonspecific toxic injury to capillaries. Farber and Sweet¹⁶ demonstrated similar membranes in the lungs of infants who had aspirated amniotic sac contents. Farber and Wilson¹⁷ also observed them in the lungs of children with lobar pneumonia or with streptococcal or tuberculous pneumonia, and they were able to reproduce the membrane experimentally in living or dead animals by the intratracheal inoculation of exudates followed by vigorous artificial respiration.

In the course of a mild epidemic of influenza that occurred in Eng-

land in December, 1936, and January, 1937, Stuart-Harris, Andrewes and Smith¹⁸ found the commonest lesion to be "bronchiolitis." Because of the relative frequency with which influenza virus was isolated from cases with such lesions, they felt that this was essentially a true influenzal condition. The fulminating pneumonias, which were relatively few in that epidemic, they felt were almost certainly due to infection with both virus and *Staph. aureus*, a combination which was demonstrated in the lungs of 3 such fatal cases. The pathological findings in 1 of the latter cases were described by Scadding¹⁹ and consisted essentially of fibrinous pleuritis, necrotizing tracheitis and bronchitis, and congestion of the lower lobes with less involvement of the apices. Histologically, there was a necrotizing alveolitis with hemorrhage filling the alveoli in infarctlike fashion. In limited areas there were small abscesses, 2 to 3 mm. in diameter; especially in these, but also throughout the affected areas, there were numerous small groups of gram-positive cocci.

Another single case in which both virus and *Staph. aureus* were isolated from the lung was reported by Stokes and Wolman.²⁰ This was a fulminating, edematous pneumonia without abscesses or hemorrhage. The patient showed a marked drop in neutrophilic leukocytes before death. The blood culture on the day before death yielded *Staph. aureus*, but culture of the heart's blood was sterile. This patient had been treated 3 weeks previously for a furuncle of the nose from which staphylococci were cultured, and there was a healing furuncle of the thigh at the time of the fatal illness.

Groups of sporadic cases of staphylococcal pneumonia not associated with definite outbreaks of influenza have been reported by a number of writers and appear to be more frequent in children than in adults.²¹⁻²⁸ In some of these cases the disease is preceded by symptoms suggesting clinical influenza, while in many the pulmonary lesions are associated with staphylococcal infection elsewhere in the body. Reimann²¹ found *Staph. aureus* to be the predominant or only organism in 10 per cent of 700 cases collected from the literature. In Habbe's autopsies,²² which are included in Reimann's series, staphylococci were found in pure culture in 7 of 93 cases of endemic bronchopneumonia and in 2 of 131 cases of lobar pneumonia, while among 20 cases of influenzal ("grippe") pneumonia they were found eight times in pure culture and five times as a mixed infection associated with streptococci, influenza bacilli, or pneumococci. Reimann²³ also described 6 cases of so-called primary staphylococcal pneumonia, 3 of which began after an influenzalike infection. All of the 6 cases presented clinical evidence of suppuration and abscess formation, but

only 2 of the patients died. The latter showed the typical pathological findings already mentioned.

Macgregor²⁴ reported autopsy findings in 10 cases of staphylococcal pneumonia in infants and children, 8 of whom were less than 1 year old. These cases occurred in one hospital during a period of 10 months. Four of the cases had early changes with massive consolidation in one or more areas of the lungs and serofibrinous pleural effusion. Hemorrhage and early onset of suppuration, especially of the bronchi and lymph vessels, were constant features. The other 6 cases were in a later stage and showed abscess formation, empyema and pyopneumothorax. Kanof, Kramer and Carnes²⁵ reported 37 cases in infants and children in which the staphylococcus was considered the etiological organism. In 25 of these patients the symptoms were similar to those of other pneumonias and the chief pathological findings were abscesses and empyema. The remaining cases had a picture predominately of sepsis with extrapulmonary foci of suppuration. There was no tendency for the cases to occur in epidemics. Cohen²⁶ reviewed some of the literature pertaining to staphylococcal infections of the respiratory tract. He estimated that in adults 1.6 to 1.8 per cent. of all pneumonias and 10 per cent of bronchopneumonias are caused by staphylococci, while 10 per cent of all primary pneumonias in infants and children are due to this organism. Infants quickly develop empyema or pyopneumothorax, while older children, like adults, develop purulent bronchopneumonia but rarely have pleural complications. Gáspár,²⁷ reviewing the autopsy material of the Rochester General Hospital for 1933-1939, found the staphylococcus to be the only or the predominant organism in 38 of 144 pneumonias (26 per cent). Twenty of these 38 patients were in the first year of life and 8 others were between 2 and 10 years old. Bronchitis and tracheitis were constant. In the newborn the picture was one of hemorrhagic lobar or lobular pneumonia. In older babies there was often gray consolidation. At times there were pin-sized to pea-sized abscesses or consolidation arranged around bronchioles. Empyema was frequent and sometimes bilateral. Melton²⁸ reported 4 cases of staphylococcal lung infections in which recovery was attributed to treatment with sulfathiazole. Interestingly enough, these cases all occurred late in January and early in February, 1941, and in one of them the history suggested that the pneumonia was a complication of clinical influenza.*

The necropsy material to be presented consists of 8 cases; 4 of these

* Since this paper was completed, there have been reported two groups of cases similar to the acute cases to be described here. It is of note that the reported cases from Boston²⁹ as well as from Durham, North Carolina³⁰ occurred during the period of the influenza epidemic which we have studied.

are considered to be acute staphylococcal pneumonia on the basis of the clinical course and pathological findings, while the remaining 4 are classified, by contrast, as chronic, or organizing, staphylococcal pneumonias. One of the acute cases had a fulminating course; death occurred 6 days after the onset of symptoms of influenza and less than 36 hours from the time when the pneumonia presumably began.* In the other 3 acute cases death occurred from 6 to 8 days after the onset of influenza and 3 to 5 days after the appearance of severe symptoms of pneumonia. One of the latter patients was 65 years old; the other 3 were in their forties. In the 4 chronic cases, the duration of the severe respiratory symptoms was estimated at 15, 22, 25 and 46 days, respectively. The longest illness was in a patient of 50 years; the other 3 patients were over 70 years of age.

BRIEF SUMMARIES OF CASE HISTORIES

Case 1. Female, 46 years old. "Influenza" began January 4th, after the patient nursed her mother who had "grippe" from January 1st to 3rd. At 10:00 a.m., January 8th, some hoarseness and fever and rhonchi were heard over the sternum. At 8:00 p.m., sudden extreme dyspnea and cyanosis. At 10:00 p.m., dullness over right lower lobe, fine râles throughout both lungs; temperature, pulse and respirations increased. Scanty blood-streaked sputum, showing gram-positive cocci. Blood culture: *Staph. aureus*. Sulfathiazole and oxygen therapy begun. January 9th at 8:00 a.m., blood culture was negative; leukocytes, 800, and erythrocytes, 4,800,000; hemoglobin, 80 per cent. At 11:00 a.m., leukocyte count, 850. Remained semicomatose after 10:00 a.m.; lungs "filled up" rapidly; respirations became increasingly rapid and shallow and the patient died at 3:30 p.m.

Autopsy was performed 1½ hours postmortem. The pleural cavities showed no excess of fluid; there were a few old basal adhesions of the right lower lobe. The right lung weighed 1125 gm., the left, 400 gm. The lungs showed a fulminating hemorrhagic pneumonia. There was slight granulocytic hyperplasia of the bone marrow. There were no other significant pathological findings. Pure cultures of *Staph. aureus* were obtained from two sites in the right lung and one in the left lung. Culture of the heart's blood showed no growth.

Case 2. Female, 43 years old. Influenza began January 7th. On January 9th, the patient had shaking chills and cough with blood-tinged sputum. January 10th, there was marked dyspnea, prostration, increasing cough and presternal soreness. January 11th, at noon, crepitant râles were heard over the left lower lobe and sonorous râles bilaterally. The temperature was 103° F. Blood culture and sputum (mouse inoculation) yielded hemolytic streptococci. Leukocytes, 14,200. On January 12th, leukocytes, 11,000. There was a rapidly downhill course with increasing dyspnea, irregular, deep respirations, livid cyanosis and extension of the signs of consolidation to involve most of the lung. Death occurred at 1:00 a.m. on January 13th.

Autopsy was performed 9 hours postmortem. The pleural surfaces showed fibrinous and fibrous adhesions radiating from the hilus posteriorly, superiorly and inferiorly, and the left pleural cavity contained 500 cc. of thin amber fluid. The pericardial cavity was completely obliterated by fibrous adhesions, very dense and adherent except along the right auricle, where they could be separated easily and

* The autopsy in this case was performed by Dr. E. B. Fisk at the Waltham Hospital, and the clinical and pathological material was made available to us through the courtesy of Dr. George E. Currier and Dr. Sidney C. Dalrymple.

offered no obstruction to the vena cava. The right lung weighed 980 gm.; the left, 700 gm. Both lower lobes and the right middle lobe contained numerous discrete and confluent acute necrotizing abscesses arranged in relation to the bronchi. There was a necrotizing bronchitis and bronchiolitis. The other organs were not remarkable. Bacteriology: heart's blood, no growth; pleural fluid and left lower lobe, *Staph. aureus* and *Streptococcus hemolyticus*; right middle and lower lobes, pus from abscess in left lower lobe and the right pleura, all yielded only *Staph. aureus*. A ferret inoculated intranasally with a suspension from this patient's lung developed no signs of infection but proved refractory to subsequent inoculation with influenza A. The ferret's serum taken prior to the second inoculation protected mice against infection with this strain of virus.¹

Case 3. Male, 46 years old. This patient had diabetes and had lost 40 lbs. during previous 8 months. January 9th, sudden onset of weakness. January 13th, slight pharyngeal injection, few crepitant and sibilant râles at the lung bases; temperature, normal; leukocytes, 6300. Diabetes readily controlled with insulin. January 15th, sudden marked prostration, dyspnea, fever and rapid pulse. Sputum culture: *Staph. aureus* predominating, and a few hemolytic streptococci. Sulfathiazole treatment begun. Dyspnea and cyanosis increased, signs in the lung extended rapidly and the patient died at 6:00 p.m., January 16th.

Autopsy was performed 17 hours postmortem. The pleural cavities contained a small amount of pleural fluid, 15 cc. in the left and 20 cc. in the right. There were fibrinous adhesions at both bases. The heart weighed 290 gm. and was normal except for slight coronary atherosclerosis. The right lung weighed 1160 gm. and the left, 960. Both lungs showed numerous acute abscesses, parenchymal hemorrhage and edema and necrotizing bronchiolitis. The liver weighed 1750 gm. and showed moderate fatty metamorphosis and minimal glycogen storage. The kidneys were pale and slightly increased in size (combined weight 420 gm.). Microscopically there was evidence of a moderate amount of glycogen storage in the tubular epithelium of the loops of Henle. Bacteriology: The heart's blood showed no growth; cultures from both lower lobes yielded a heavy growth of *Staph. aureus* and a few alpha hemolytic streptococci.

Case 4. Male, 65 years old. Influenza with cough and scanty sputum began January 6th. Cough increased January 9th and was accompanied by pain in the right side of the chest. January 12th, sudden marked dyspnea. January 13th, at 9:00 p.m., intense cyanosis, restlessness and air hunger. Areas of consolidation in both lungs, more on the right, with numerous scattered rhonchi. Temperature, 103.4° F.; pulse, 120; blood pressure, 170/80; blood culture yielded no growth. The patient died at 2:00 p.m. on January 14th.

Autopsy was performed 2 hours postmortem. The pleural surfaces of both lungs were covered with fibrinous exudate. There were 700 cc. of turbid yellow fluid in the right pleural cavity and 75 cc. of similar fluid in the pericardial cavity. The heart weighed 460 gm. and showed slight hypertrophy of the myocardium of both ventricles. Histologically, there was no evidence of pericarditis, nor did the heart muscle appear remarkable. The right lung weighed 1000 gm. and the left, 600. There was a healing infarct 3 by 4 cm. in diameter in the right upper lobe. The lungs showed hemorrhage, edema, necrotizing bronchitis and abscess formation. The liver weighed 2450 gm. and showed moderate central congestion. *Staph. aureus* was grown in pure culture from the lungs, and from the pleural and pericardial fluids.

Case 5. Female, aged 74 years. Admitted on January 12th, irrational. Severe dry cough since January 5th. There was Paget's disease of the skull, emphysema and auricular fibrillation. Blood pressure, 160/100. The patient was digitalized. There was an irregular low-grade fever. Leukocytes, 11,600. Sputum culture: *Staph. aureus*, type 17 pneumococcus and alpha streptococci. January 17th, notwithstanding signs of bilateral bronchopneumonia, the patient's condition was improving and

she was sitting up in bed. On January 19th, the patient again became irrational and thereafter the course was rapidly downhill with increasing dyspnea.

Autopsy was performed 45 hours postmortem. There were a few yellow-gray fibrinous bands on the pleura over the right upper lobe and some fibrinous patches over the lateral surfaces of both upper and lower lobes. The heart weighed 300 gm. and showed minimal atherosclerotic changes. The right lung weighed 770 gm., and the left, 500. Microscopically, there was evidence of pulmonary fibrosis with areas of walled-off abscess cavities connected with dilated bronchi. The process was most marked in both lower lobes posteriorly. The liver weighed 1400 gm. and showed slight engorgement of the central veins. The combined weight of the kidneys was 260 gm. and they showed a few scattered scars of healed pyelonephritis. The calvarium was thickened to 1.3 cm. and showed characteristic changes of Paget's disease; these changes were also seen in the vertebral column.

Case 6. Female, age 76 years. This patient was admitted January 24th with a history of a "cold," marked weakness and chest pain since January 18th. There were signs of consolidation of the right lung anteriorly, and extrasystoles. Blood pressure, 130/80. Sputum yielded *Staph. aureus* in pure culture; two blood cultures were negative. Leukocytes, 24,400. Treatment was with sulfadiazine for 6 days; digitalis was given in full doses. Temperature and leukocytes fell to normal by January 28th. February 3rd, leukocytes, 5500; irregular fever; dyspnea, and cyanosis. Râles in lungs increased progressively and patient died February 9th.

Autopsy was performed 18 hours postmortem. The right pleural cavity was obliterated by diffuse fibrous adhesions; scattered fibrinous adhesions were found in the left pleural cavity laterally. Heart weighed 260 gm. and showed minimal atherosclerotic changes. The right lung weighed 510 gm.; the left, 350 gm. Firm gray areas of fibrosis were found in the right upper and middle lobes, and moderate bronchiectasis in both lower lobes. Microscopically, there was moderate fibrosis of the lung parenchyma and moderately severe bronchiectasis. There were multiple benign polyps in the descending colon and rectosigmoid. There was microscopical evidence of moderate benign nephrosclerosis and a few scattered areas of healed pyelonephritis. Culture from the lungs showed *Staph. aureus* and influenza bacilli; the heart's blood showed no growth.

Case 7. Female, 50 years old. "Grippe," coryza, weakness and cough began December 24th. December 30th, sudden marked dyspnea and air hunger. Diffuse crepitant and sibilant râles and scattered areas of dullness and bronchial breathing at the bases. Irregular fever to 103° F., leukocytes 9000 to 18,000, constant and increasing dyspnea and cyanosis. Treatment with sulfathiazole, digitalis and oxygen was begun on admission and continued until the patient died on February 18th. She also received many small transfusions. Many sputum cultures showed *Staph. aureus* predominating in all specimens; type 14 pneumococcus was also obtained in the first specimen and hemolytic streptococci in the last two. Numerous blood cultures were negative except one taken on February 7th, which showed *Staph. aureus*.

Autopsy was performed 14 hours postmortem. There were bilateral fibrous pleural adhesions. The heart weighed 440 gm. The right auricle and ventricle were dilated and the latter showed a slight increase in thickness of the myocardium (0.5 cm.). Microscopically, miliary abscesses were seen in the myocardium. The left lung weighed 930 gm. and the right, 940. Both showed extensive fibrosis, bronchiectasis and thick-walled abscesses in the parenchyma, involving most of the lungs but especially marked posteriorly and inferiorly. The spleen weighed 500 gm. and contained a few small scattered abscesses. The liver weighed 2400 gm. and contained small abscesses in the portal and midzonal areas of the lobules. The combined weight of the kidneys was 340 gm. Microscopically, occasional glomeruli and neighboring collecting tubules contained acute inflammatory exudate and

there was an occasional small interstitial abscess in the pyramids. The other organs were negative. Cultures from many areas in the lungs showed *Staph. aureus* and hemolytic streptococci in varying proportions, and the heart's blood showed no growth.

Case 8. Male, 81 years old. Ill 2 to 3 weeks with prostration and vomiting. Admitted January 26th. Signs of diffuse bronchopneumonia, mostly in right lower lobe; temperature, 104° F.; pulse, 140, and leukocytes, 10,000. Sputum yielded a heavy growth of *Staph. aureus* and a few alpha hemolytic streptococci. Blood cultures were negative. There was improvement with drop in temperature and pulse rate after 2 days of therapy with sulfadiazine, digitalis and oxygen. February 1st, sudden rise in temperature, pulse and respiration; leukocytes, 27,000. The patient died February 3rd.

Autopsy was performed 11 hours postmortem. There were diffuse fibrous adhesions of both pleural cavities. The heart weighed 240 gm. and showed minimal atherosclerotic changes. The right lung weighed 500 gm. and the left, 560. The posterior lobes of both lungs and the right middle lobe showed considerable fibrosis of the parenchyma and bronchiectatic cavities. Acute inflammatory exudate was present in focal areas, especially about cellulose material in terminal bronchi. There was early organization of the exudate with foreign body giant cells in scattered areas, especially about the cellulose material. There was an old area of fibrosis at the right apex but no evidence of active tuberculosis. The kidneys showed the changes of benign nephrosclerosis. Cultures from both lower lobes yielded *Staph. aureus* and alpha hemolytic streptococci.

ACUTE STAPHYLOCOCCAL PNEUMONIAS

The findings in the lungs of 3 of the acute cases were essentially similar. In them the involvement was bilateral and included portions of all the lobes, but it was most marked in the posterior and dependent portions. The lungs were increased in size and weight; the right lung varied from 980 to 1160 gm., the left from 600 to 960 gm. There was no definite exudate over the pleural surfaces in any of the cases, but in 2 of them there was a moderate amount of thin fluid in one pleural cavity. In 1 there were 500 cc. of clear amber fluid and in the other there were 700 cc. of fluid described as turbid yellow. Both these fluids yielded *Staph. aureus* on culture. In all 3 cases there were minimal fibrous adhesions at the posterior apical region, and in 1 case there were thin intralobar fibrinous strands. There was no evidence of subpleural hemorrhage, nor were any emphysematous blebs observed.

The lungs completely filled the pleural cavities and maintained their shape when they were removed from the thorax. Externally they presented mottled dull gray to dark red-purple anterior surfaces, which gradually blended into plum-colored and mottled posterior surfaces. In 1 case (Fig. 1) there was an area of old infarction in the right upper lobe which measured 3 by 4 cm., appeared dark red and had a uniform rubbery consistence on palpation. Scattered throughout the posterior lobes and in the inferior portion of the anterior lobes were

numerous gray areas which felt nodular and varied from 0.5 to 3.0 cm. in diameter. On the cut surface these nodular areas presented a homogeneous dirty gray appearance and exuded a mucoid exudate. When this exudate was scraped away, multiple abscess cavities were revealed which varied from 2 to 5 mm. in diameter, many coalescing to form honeycombed cavities measuring 1 to 2 cm. in diameter (Fig. 2). These abscessed areas were arranged, in general, in relation to the bronchi and bronchioles and usually were in direct communication with them. There was no evidence of any well defined wall to these abscesses, the contiguous parenchyma appearing markedly necrotic and fading into apparently intact alveoli filled with exudate. The intervening parenchyma presented a varied appearance; in some areas it was extremely wet and subcrepitant and exuded frothy bloody fluid, while in others the gray exudate-filled alveoli could be distinguished. Most of the parenchyma was involved and only about one-fifth to two-fifths of the lung was estimated to be air-containing. The trachea and bronchi were filled with plugs of yellow-gray, tenacious exudate. The pulmonary arteries and veins appeared grossly normal.

Sections were taken from various parts of the lungs for microscopical examination. There was extensive destruction of the usual architecture by an acute necrotizing process which was most marked in and about bronchi and bronchioles and involved adjacent alveolar tissue. The bronchi and bronchioles were almost completely denuded of their mucosa and their walls were infiltrated with acute inflammatory cells, chiefly polymorphonuclear leukocytes and histiocytes, and an abundance of fibrin (Fig. 7). The lumina of the ducts were filled with an acute inflammatory exudate. In 2 of the 3 acute cases there was no evidence of a hyaline membrane such as was so prominent in the influenzal pneumonias of 1918. The inflammatory process extended into the subtending alveoli with necrosis of the septa and resulted in the formation of abscesses, which varied in size from small miliary abscesses to large, conglomerate, necrotic masses (Fig. 6). There was no evidence of any limiting abscess wall of young connective tissue, nor was there any evidence of organization of the exudate. In some sections large veins were seen projecting into the abscesses. These veins were infiltrated with inflammatory cells and some of them contained fibrin thrombi in their lumina. Elsewhere, the blood vessels were markedly engorged, and in some areas they had actually ruptured and filled the alveoli with blood (Fig. 5). Other alveoli contained large amounts of edema fluid but no blood.

Large numbers of cocci in groups and clusters were seen in the exudate in scattered areas (Fig. 8). In case 2, smaller cocci in short

chains were also seen. The latter, however, were not present in any great numbers in the abscessed areas which showed the clumps of cocci. They were seen chiefly in alveoli which were filled with exudate but which had maintained their usual structure.

The pathological picture in the lungs in case 1 was somewhat different from that seen in the other 3 acute cases, and corresponded more closely to that of the most fulminating variety of influenzal pneumonia described in 1918. In this case the process involved chiefly the right lower and middle lobes and a small area in the left lower lobe. Grossly, the involved lobes were noncrepitant, heavy and moist, and exuded an abundance of bloody fluid. Microscopically, the picture in these lobes was predominantly one of hemorrhage and edema with very little fibrin and only a few polymorphonuclear leukocytes. Nevertheless, there were many groups of cocci seen. In spite of the meager leukocytic response, there was necrosis of alveolar walls. A few alveoli contained suggestive hyaline membranes. The bronchi showed marked edema and necrosis with very little cellular reaction. In uninvolved areas the alveoli were distended and some were disrupted, forming emphysematous blebs.

Cultures from the lungs yielded an abundant growth of *Staph. aureus* in every instance. In case 2, hemolytic streptococci were grown, in addition, from one of three lobes cultured, and a few alpha hemolytic streptococci were grown in case 4. Ferret inoculation of lung tissue from case 2 gave indirect evidence of the presence of influenza A virus.

The only other relevant finding in the acute cases was in the pericardial cavity in case 4. This contained 75 cc. of yellow turbid fluid, from which a pure culture of *Staph. aureus* was obtained. Microscopically, however, there was no evidence of acute pericarditis. Other incidental findings included an old obliterative fibrous pericarditis in case 2 and cardiac hypertrophy involving both ventricles in case 4. Cultures of the blood were made at the time of autopsy in every case and all remained sterile.

CHRONIC STAPHYLOCOCCAL PNEUMONIAS

Four cases presented pathological findings which were similar, in most respects, to those of the "organizing" pneumonias described by Winternitz, Wason and McNamara¹⁰ and others in relation to the influenza of 1918. The general appearance of the lungs was more or less alike in these cases. The weight of the lungs varied greatly: the right weighed from 510 to 940 gm. and the left from 350 to 930 gm. There were fibrous adhesions bilaterally, and these were moderately

extensive posteriorly, but there were no areas with exudate or fibrin. The pleural surfaces of the lungs presented a mottled appearance varying in color from a dark gray-red in the posterior portions to a gray-pink in the areas of emphysema which were most prominent in the upper lobes and in the periphery of the lungs. On palpation the basal portions of the lungs contained firm, rubbery nodular areas, especially posteriorly (Fig. 3). The upper lobes at their bases presented a similar, but less pronounced nodular change. In 1 case the major portion of the involvement was limited to the right upper and middle lobes; the right lower and entire left lung were only slightly involved.

On section the nodular areas were seen to be composed of large cavities with firm, well demarcated fibrous walls (Fig. 4). The majority contained yellow or gray-green mucoid exudate which could be expressed from the cavities. Most of the cavities communicated with bronchi which, in turn, were plugged with similar exudate. There was extensive fibrosis between the cavities, where the usual architecture was almost completely replaced by fibrous tissue. In areas which did not contain cavities the tissue either appeared emphysematous or was firm and of a dull red-gray color. There was no evidence of hemorrhage in the parenchyma. The cut surface was moderately dry. Trachea, bronchi and bronchioles contained gray-green, tenacious exudate which, when removed, revealed an apparently intact but erythematous mucosa. The pulmonary arteries showed no evidence of atheromatous intimal plaques, nor were they dilated. The pulmonary veins were not remarkable.

Microscopically there was evidence of extensive fibrous replacement of the alveoli. Large abscesses were in direct continuity with bronchi, forming bronchiectatic cavities. The bronchial walls were infiltrated by numerous plasma cells, scattered lymphocytes and histiocytes and rare foreign body giant cells (Fig. 9). The abscess cavities were filled with a more acute inflammatory exudate, and their walls showed some persistence of the necrotizing process. In 1 case some recently aspirated cellulose material was seen. In 3 of the cases there was an interstitial fibrosis in addition to chronic abscess cavities. The alveoli in the areas of interstitial fibrosis were lined by low cuboidal epithelium. There was evidence of regeneration of the bronchial mucosa (Fig. 10). A number of areas of regenerating mucosa appeared to be pinched off and imbedded in the contiguous parenchyma, forming small islets of epithelial cells. A similar regenerative process was described by Winternitz, Wason and McNamara¹⁰ in the cases observed in the 1918 influenza epidemic.

The alveoli in some areas contained inflammatory exudate in different stages of organization. Large sheets of young connective tissue could be seen extending from one alveolus into another through septal defects. The alveolar capillaries were all dilated and filled with blood, as were the majority of the veins. The arteries in the areas where there was marked fibrosis and chronic inflammation showed a very early proliferative endarteritis, but no evidence of necrotizing arteritis.

In the cultures made at autopsy, *Staph. aureus* was the predominant organism recovered from the lung in all the cases. Alpha hemolytic streptococci were present in small numbers in 2 cases, beta hemolytic streptococci occurred in another and influenza bacilli were grown from 2 cases. The heart's blood showed no growth in any case.

The cardiac weights were within average range. There was a slight thickening of the right ventricular myocardium in case 7. The myocardium in this case contained small acute abscesses which were also seen in the spleen, liver and kidneys, suggesting blood-borne metastases.

The vertebral bone marrows in both the acute and chronic cases showed hyperplasia of both the red and the white blood cell series with normal maturation of the cells.

COMMENT

The pathological findings in these cases are similar, in most respects, to those described in the influenzal pneumonias of 1918. All stages are represented—the fulminating and the necrotizing varieties of the acute form and the organizing type in which death was delayed for longer than 2 weeks.

The cases presented are of interest mainly for two reasons: First, they represent the severest of the pneumonias that may be observed in the course of an epidemic of influenza which, from all other points of view, could be considered as comparatively mild. The first symptoms of respiratory infection in all these cases occurred within a period of 4 weeks, from December 24, 1940, (case 7) to January 18, 1941, (case 6), during which influenza was prevalent in and around Boston. In 5 of the cases there were typical symptoms of clinical influenza which began within a single week, between January 3rd (case 6) and January 9th (case 3), when the epidemic was at its peak.

Secondly, these cases are of special interest because they show clearly the important rôle played by *Staph. aureus* in the pulmonary complications of this epidemic. Other cases of pneumonia that occurred during the same period were of the usual varieties, both clinically and pathologically, and they were associated with the usual organisms

encountered in that season. Probably some of the latter patients also had clinical influenza, or at least contact with influenza virus, as indicated by immunological studies.¹ The staphylococcus, however, was unusually prevalent as an apparently important secondary invader in some cases of typical pneumococcal pneumonia that occurred at this time. It was the only or predominant organism in a large number of patients with clinical influenza, many of whom had pulmonary complications. Those among the latter who survived presented a variety of clinical pictures which have been described briefly elsewhere;² the pathological lesions in the fatal cases have been described here. The clinical features have been described in greater detail recently.³¹

In the experience of this hospital, staphylococcal pneumonias have been observed sporadically, especially in infants and young children and as a complication of staphylococcal infection elsewhere in the body. This is the first time that the staphylococcus has been identified in relation to a large number of apparently primary pulmonary infections within such a brief period.

SUMMARY AND CONCLUSIONS

The pathological findings in 8 cases of pneumonia in which *Staph. aureus* was the only or predominant organism have been presented. These cases all occurred during an epidemic of influenza and probably represent pulmonary complications of influenza. Three forms similar to those described among the influenzal pneumonias of 1918 were included; namely, (1) the acute fulminating, (2) the acute necrotizing and (3) the chronic organizing types. From this experience and from a number of reports in the literature, it is suggested that during an epidemic of influenza *Staph. aureus* may assume epidemic prevalence in some areas and give rise to severe pulmonary complications.

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DESCRIPTION OF PLATES

PLATE 5

- FIG. 1. Left lung (case 2) showing extensive diffuse hemorrhage, edema and exudate. Confluent peribronchial abscesses are present in the lower lobe.
- FIG. 2. Detail view of part of the lung shown in Figure 1. The major bronchi show acute confluent and necrotizing bronchitis.
- FIG. 3. Left lung (case 7) showing marked distortion from extensive fibrosis and slight emphysema.
- FIG. 4. Detail view of base of lower lobe of the lung shown in Figure 3. There is replacement of the usual structure by fibrous tissue and "honeycombed" cavities..

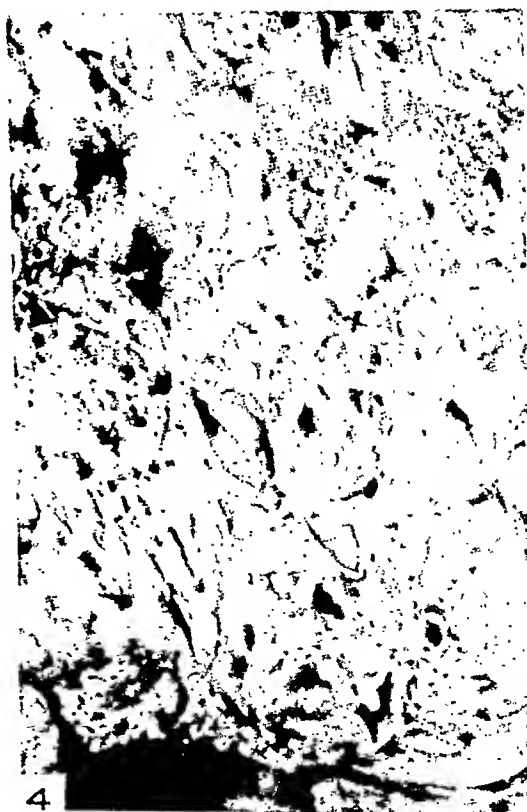
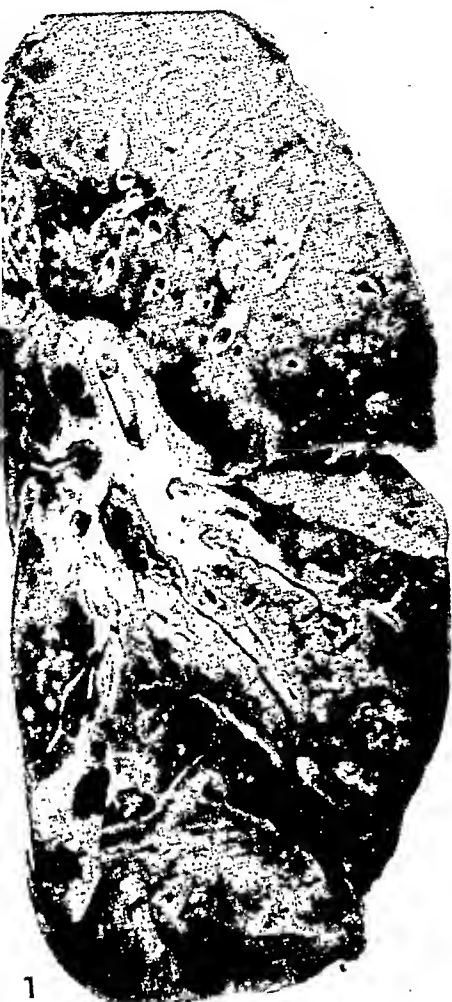
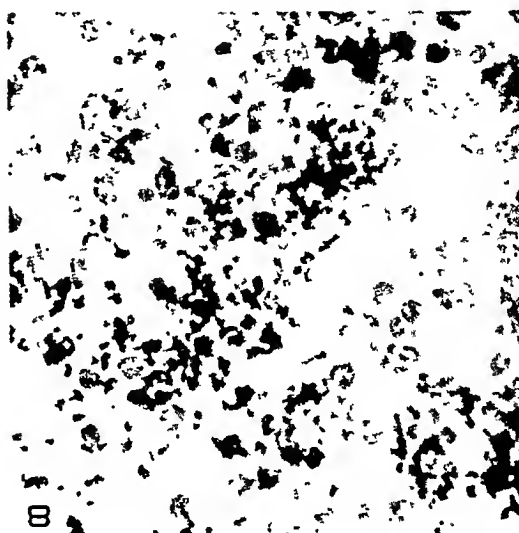
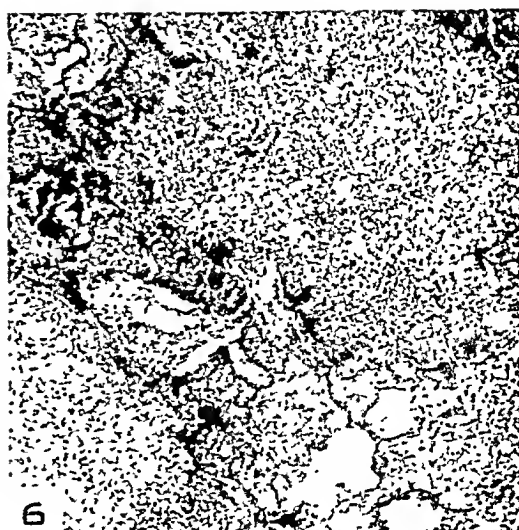
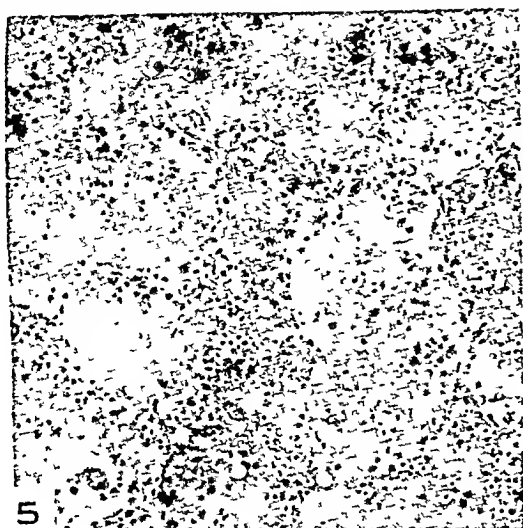


PLATE 6

- FIG. 5. (case 2). Acute hemorrhage and edema fluid filling alveoli and destroying alveolar walls. Minimal exudate. Phloxine-methylene blue stain. $\times 70$.
- FIG. 6. (case 3). Acute abscesses of lung parenchyma. Here may be seen an hyaline membrane lining the alveoli in an area of trapped air. Phloxine-methylene blue stain. $\times 50$.
- FIG. 7. (case 2). Acute necrotizing bronchitis. Phloxine-methylene blue stain. $\times 100$.
- FIG. 8. (case 2). Large clumps of cocci in acute exudate. Gram-Weigert stain. $\times 750$.
- FIG. 9. (case 7). Extensive fibrous tissue replacement of lung tissue resulting in multiple small cavities. Phloxine-methylene blue stain. $\times 100$.
- FIG. 10. (case 6). Proliferation of bronchial epithelial mucosa in areas of extensive fibrosis. Phloxine-methylene blue stain. $\times 100$.



CHRONIC GASTRITIS

ITS RELATION TO GASTRIC AND DUODENAL ULCER AND TO GASTRIC CARCINOMA*

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The term, gastritis, is commonly used in a restricted sense to designate a series of changes manifested chiefly in the mucosa of the stomach. Histologically, the process is characterized for the most part by excessive accumulations of lymphocytes and plasma cells and by atrophy and abnormal regeneration of the glands. These and other features have been described in detail by numerous writers as they occur in relation to gastric and duodenal ulcer and carcinoma of the stomach as well as in relation to gastritis alone (Konjetzny,¹ Faber²). The significance of the findings has been minimized, however, by reports of a high incidence of the same anatomic changes in individuals free of ulcer or carcinoma (Hamperl,³ Hillenbrand⁴). The uncertainty and largely speculative consideration of etiologic factors emphasize that doubted significance.

A relatively large number of resected stomachs which presented the features of chronic gastritis in addition to the major lesion—ulcer or carcinoma—for which resection was performed stimulated interest in the problem. This material offered an opportunity to verify or disprove the character and distribution of the reported mucosal changes in relation to ulcer and carcinoma. In view of the reported high incidence of the same changes in otherwise normal stomachs, it was considered necessary to study a series of stomachs from patients free of manifest gastric disease. In this paper the findings in the surgical material will be reported and compared with the findings in control autopsy material.

MATERIAL AND METHODS OF INVESTIGATION

A. Autopsy Material

The stomachs of 260 individuals free of manifest gastric disease served as controls. This material was obtained from autopsies performed by members of the Department of Pathology at the University of Minnesota, and the great majority were from the autopsy service of the University of Minnesota Hospitals. These were patients whose histories revealed no gastric symptoms except as related to the pre-

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senting illness and were otherwise unselected, except in so far as the autopsy experience may deal with selected cases. The reliability of the recorded historical data may be questioned in some instances, but it may be said that at least there were no serious gastric disorders among the group.

Microscopic sections were prepared from the lower antrum near or including, in most instances, the pylorus and from the midbody on or near the greater curvature in all cases. In addition sections were frequently taken from the upper antrum on the lesser curvature and from other portions of the body. Only specimens free from obscuring postmortem changes were used. Whether sampling of this kind adequately depicted the condition of the mucosa as a whole was tested by study of 30 specimens in greater detail. In these, long strips of mucosa were prepared according to the method used routinely for the surgical material (see below). These were compared with the sample sections. They showed the same features and no case had to be reclassified. While undoubtedly some focal changes may be missed, the sampling seems to provide a reasonably accurate expression of the condition of the antral and body mucosa, at least for stomachs free of ulcer or carcinoma. If focal changes were missed, there was an equal chance of picking up focal lesions in the sections examined, which, in terms of significant change, is the more serious error.

Stigmata of gastritis were searched for in each specimen and the findings were recorded separately for the antrum and body. For the purposes of this study, only the principal anatomic features of chronic gastritis were recorded; *viz.*, lymphocytic and plasma cell infiltration, lymph follicles, atrophy of the glands, metaplasia to an intestinal mucosal pattern and, additionally for the body mucosa, the pseudopyloric glands (Stoerk⁵). The degree of change for each of these features was arbitrarily graded from 1 to 3 and the specimens were divided into three groups on the basis of the following criteria:

Group 1. Antrum. The glands are regularly distributed, free from atrophy and of metaplasia to intestinal type. The stroma is scant and free, or almost free, from lymphocytes and plasma cells other than a rare basilar lymphoid aggregate with or without a germinal center (Fig. 5).

Body. The glands are regularly arranged and intact. The stroma contains no, or very few, free cells in the interfoveolar areas (Fig. 1).

Group 2. Antrum. The glands show the same features as those of group 1. The stroma shows mild lymphocytic and plasma cell infiltration (grade 1) and rare lymphoid follicles (grade 1) (Fig. 6).

Body. The glands are the same as in group 1. The stroma shows

mild superficial lymphocytic infiltration (grade 1) and not more than an occasional follicle (grade 1) (Fig. 3).

Group 3. Antrum. Here the changes are more variable. Moderate lymphocytic and plasma cell infiltration alone (grade 2), atrophy (grade 1 to 3) alone, or metaplasia (grade 1 to 3) alone was sufficient to place a case in this group. The great majority of specimens showed combinations of stromal and glandular changes (Figs. 7 and 11).

Body. Although moderate lymphocytic and plasma cell infiltration (grade 2) alone placed a case in this group, the majority showed associated glandular changes in the form of atrophy, grade 1 to 3, or metaplasia, grade 1 to 3. Moderately severe infiltration was seldom seen in the absence of glandular changes (Figs. 2, 4 and 8).

B. Surgical Material

The surgical material included in this study consisted for the most part of stomachs excised for ulcer or carcinoma. A few cases in which the excised specimen showed only benign polyps or atrophic gastritis were also included. Except for the removal of long segments from the greater curvature in 8 resections of the fundus, extensive gastric resection had been employed in all instances.

The gross specimens were routinely opened on the greater curvature, tacked to a board and floated in a 4 per cent solution of formaldehyde for fixation. Except in a few instances, fixation was begun within 10 minutes from the time the specimen was removed from the patient. In dealing with duodenal ulcer the surgical technic employed frequently left the ulcer in the inverted duodenal stump. In such cases the diagnosis of duodenal ulcer was accepted on the basis of the roentgenologic and surgical findings.

The usual histologic sections from selected areas were studied in all cases. In addition, rolled strips of mucosa from the whole length of the segments excised were prepared for section as follows: The mucosa was incised in parallel lines about 1 cm. apart and the intervening strip was separated from the muscularis propria by sharp dissection. Segments of these strips of convenient length were then rolled up, trimmed to a thickness of about 3 mm. and paraffin sections were prepared in the usual manner. In many instances segments up to 15 cm. long could be embedded in a single roll, but as many rolls were prepared as the length of the strip required. Such rolls were routinely prepared from both the greater and the lesser curvatures. In some instances additional rolls from the anterior and posterior walls were employed or these sites were substituted for the curvatures. Following resections of the fundus, rolls were prepared from the greater curva-

ture only. This method of study was apparently first employed by Hallas⁶ and has been used by Hillenbrand,⁴ Geissendörfer,⁷ Magnus⁸ and others.

The same abnormal features were noted in the surgical material as in the autopsy material (Figs. 2, 3, 4, 6, 7 and 8). For the body mucosa, "extent," indicating the proportion of the segment examined which was involved by the above changes, was graded 1 (not more than $\frac{1}{3}$), 2 ($\frac{1}{3}$ to $\frac{2}{3}$), or 3 (total). In addition to the three groups designated according to the several criteria as for the autopsy cases, a fourth

TABLE I
Summary of Findings, as Graded, in the Antral Mucosa of Cases Examined after Autopsy

Age	Infiltration			Follicles			Atrophy			Metaplasia			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Under 1	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	16
1-10	0	1	0	1	0	0	0	0	0	0	0	0	26	0	1	27
11-20	5	0	0	3	1	0	0	0	0	1	0	0	6	4	1	11
21-30	3	2	0	1	1	0	0	0	0	0	0	0	19	3	2	24
31-40	10	4	2	4	0	1	5	0	0	1	0	0	31	9	7	47
41-50	8	2	0	5	0	0	2	0	0	1	0	0	17	7	3	27
51-60	11	7	0	3	1	0	6	1	1	4	1	2	17	9	10	36
61-70	17	4	1	2	2	0	4	4	1	3	3	3	15	11	11	37
71-80	10	3	2	3	0	0	4	3	1	2	4	0	13	6	8	27
81-90	3	2	0	0	0	0	2	1	1	2	1	2	2	1	5	8
Totals	67	25	5	22	5	1	23	9	4	14	9	7	162	50	48	260

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

group was added for the cases with ulcer to accommodate instances in which the mucosa was hypertrophic.

GASTRITIS IN POSTMORTEM MATERIAL

For the entire group of 260 cases examined after autopsy the findings are summarized in Tables I and II for antral and body mucosa respectively. Each case was given a group designation of 1, 2, or 3 depending on the severity of the changes. Group 1 consisted of those cases which by all accepted criteria are normal. Group 2 cases were those which showed mild lymphocytic and plasma cell infiltration without parenchymal change. Although this appearance was a deviation from the usually accepted normal, it is doubtful that the degree of change exhibited is of real significance. Group 2, then, was arbitrarily designated as "almost normal." Distinctly abnormal findings placed a specimen in group 3 and these were so designated on the basis of moderate infiltration alone, or in any degree when accompanied by glandular changes. It is obvious that there is no sharp dividing line between the several degrees of lymphocytic infiltration. Thus the distinction between "mild" and "moderate" is necessarily arbitrary.

Several independent groupings of the material, however, yielded uniform results.

In respect to the antrum, 162 specimens (62.2 per cent) fell in group 1 (normal), 50 (19.2 per cent) fell in group 2 (almost normal) and 48 (18.5 per cent) were distinctly abnormal. As shown in Table I, the abnormal cases were scattered through the several decades, but increased in incidence after the age of 30 years. These findings may be compared with the findings in cases with ulcer and with carcinoma, to be discussed later, in Table IX.

TABLE II
Summary of Findings, as Graded, in the Body Mucosa of Cases Examined after Autopsy

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
Under 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	16
1-10	1	1	0	4	0	0	0	0	0	0	0	0	0	0	0	25	1	1	27
11-20	2	0	0	4	0	0	0	0	0	0	0	0	0	0	0	9	2	0	11
21-30	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	19	4	1	24
31-40	9	7	1	2	1	0	1	1	0	1	0	0	1	0	0	30	9	8	47
41-50	3	2	1	2	0	0	0	0	0	0	0	0	0	0	0	21	3	3	27
51-60	3	12	4	3	1	0	3	6	3	3	3	0	6	0	1	17	3	16	36
61-70	15	4	2	1	0	0	3	3	5	6	2	2	4	2	0	16	6	15	37
71-80	2	4	4	2	0	0	2	2	2	3	1	1	2	1	0	17	2	8	27
81-90	1	3	1	0	0	0	1	1	2	0	1	1	0	1	0	3	0	5	8
Totals	40	34	13	19	2	0	10	13	12	13	7	4	13	4	1	173	30	57	260

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

For the body mucosa (Table II) 173 specimens (66 per cent) fell in group 1 (normal), 30 (12 per cent) fell in group 2 (almost normal) and 57 (22 per cent) fell in group 3 (gastritis). Of the 57 cases in group 3, all but 2 were over 30 years old, and of those which showed atrophy, all but 2 were over 50 years of age. Atrophic gastritis was present in 30 per cent of the 108 cases past 50 years of age. The findings in the body mucosa of this group may be compared with those of the surgical material in Table X.

The findings in antrum and body were not always parallel. In 13 of the specimens in which the antral mucosa was distinctly abnormal the body was normal or almost normal. These cases were scattered uniformly through the several decades. Twenty-one specimens showed a normal or almost normal antrum and a distinctly abnormal body. These appeared according to decades as follows: fourth decade, 3; fifth, 2; sixth, 8; seventh, 6; eighth, 2.

In none of the cases which revealed abnormalities was there any evidence of a causative factor such as the various exogenous and endogenous irritants to which the changes have been attributed by Faber² and others. For the entire group the findings were independent

of the fatal illnesses and their durations. There was no greater incidence of gastritis in individuals who died of infectious diseases. In so far as the recorded histories yielded information, there was no demonstrable relationship to previously sustained diseases. There were no sex differences. The only constant factor was that of age. Gastritis was rarely encountered below the age of 30, and of those specimens which showed atrophic changes only 2 appeared in individuals below the age of 50 years. In terms of this autopsy experience the high incidence of gastritis in adult stomachs reported by Hamperl,³ Hillenbrand⁴ and others cannot be substantiated, at least for individuals below 50 years of age. It appears that the majority of the stomachs obtained at autopsy are free of significant change.

SIMPLE GASTRITIS IN RELATION TO GASTRIC AND DUODENAL ULCER

Since the early 1920's numerous papers have appeared emphasizing the constancy of an inflammatory process involving particularly the antrum in cases of both gastric and duodenal ulcer (Moszkowicz,⁹ Konjetzny,^{1,10,11} Kalima,¹² Orator,¹³ Puhl,¹⁴ Borchardt,¹⁵ Puchert,¹⁶ Aschner and Grossman,¹⁷ Simpson,¹⁸ Magnus and Rodgers¹⁹ and others). These studies covered a large volume of material and in general the same pattern of mucosal alteration was common to all—an antral gastritis showing variably heavy lymphocytic infiltration, abnormal numbers of lymphoid follicles, atrophy and acute and chronic erosions. These same investigators reported an equally high incidence of duodenitis in their material. Changes in the body mucosa have not been as systematically investigated, particularly in respect to their topographic distribution. The reports indicate a lower incidence of gastritis involving the body mucosa in duodenal ulcer than in gastric ulcer. So-called hypertrophic gastritis is not infrequently associated with ulcer but there are few direct references to its incidence in resected material.

The findings in 106 stomachs resected for ulcer (78 duodenal, 13 gastric and duodenal, and 15 gastric) are recorded in this paper.

The antral changes rather closely paralleled those described in other reports. The gross appearance of the antral mucosa varied rather widely. Color changes were inconstant and, as congestion and areas of fresh hemorrhage dependent on surgical trauma precluded accurate observation, this feature was generally ignored. Exaggerated irregularity of the *areae gastricae* was not uncommon. Frequently part of the antral mucosa appeared to be more thin and smooth than normal. Such areas were most commonly seen at the apex of the antral triangle. In some instances the atrophy was diffuse but left, here and there,

islands of mucosa of more normal thickness. Grossly demonstrable erosions were infrequent. Only 2 specimens presented the typical appearance of the erosive gastritis illustrated by Konjetzny,¹ Faber,² Puhl¹⁴ and others. In a few specimens one or two erosions were demonstrable.

The body mucosa regularly showed no gross changes of significance except in those instances where it could be recognized as hypertrophic.

As in the material obtained at autopsy, the histologic changes were recorded separately for antral and body mucosa. Such alterations were

TABLE III
Summary of Findings, as Graded, in the Antral Mucosa of Cases with Ulcer

Age	Infiltration			Follicles			Atrophy			Metaplasia			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
11-20	1	1	0	0	2	1	1	0	1	0	0	0	0	0	0	2
21-30	0	6	2	2	5	0	3	3	1	0	0	0	0	0	0	8
31-40	2	20	4	9	12	5	10	10	2	1	2	0	0	0	0	26
41-50	2	19	5	8	10	8	11	11	3	6	1	1	0	0	0	26
51-60	2	22	3	7	16	4	6	15	6	9	1	0	0	0	0	27
61-70	0	7	0	2	4	1	0	3	3	3	0	0	0	0	0	7
71-80	1	1	0	0	1	1	0	2	0	2	0	0	0	0	0	2
Totals	8	76	14	28	50	20	31	44	16	21	4	1	0	0	98	98

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

admirably brought out by means of the mucosal rolls inasmuch as there were often variations in distribution and severity in the antrum. The changes were quite regularly more pronounced on the lesser than on the greater curvature. The antral findings in 98 cases with ulcer are summarized in Table III. The specimens from 8 resections of the fundus, which yielded little or no antral mucosa, were excluded. As shown in the table, there were no specimens in which the antrum was normal or almost normal. All showed distinctly abnormal features and fell in group 3.

Not included in the tabulations are acute erosions which were found in but 3 specimens. Erosions were otherwise chronic, usually healed, occasionally healing. Microscopic erosions of this character were found in 25 per cent of the cases (Fig. 11).

The antral mucosa was considered to be hypertrophic in 19 specimens (not indicated in the tables). Seventeen of these were in the group with duodenal ulcers, 6 associated with hypertrophic body mucosas and 11 without. Two were found in the group with combined duodenal and gastric ulcer, 1 of which had an associated hypertrophic body mucosa. None was found in the group with gastric ulcers. The upper limit of normal thickness is not well defined. Plenk²⁰ considered a thickness of 1.5 mm. as normal, while Berger's²¹ data indicated an

average thickness of about 0.65 mm. In my autopsy material the thicknesses in adults ranged around 0.80 to 0.90 mm. A mucosa of 1.25 mm. is thicker than any encountered in the autopsy group or in the bulk of the surgical material, although this level was approached more frequently among the surgical specimens. Most of the specimens designated as hypertrophic were 1.5 mm. or over (Fig. 10). All of these had superimposed inflammatory changes and all showed areas where destruction and atrophy had occurred. It should be emphasized that hypertrophy as it is here considered means an increase in all constituents of the mucosa so that both glands and crypts are elongated while their proportionate relationships to mucosal thickness are maintained. Relative or absolute increase in the length of the crypts at the expense of the glands proper—atrophic hyperplastic changes—are not included.

While this study is not primarily concerned with the condition of the duodenal mucosa, the findings in this series of cases may be mentioned. Inasmuch as little or no duodenal mucosa was included with many specimens, a detailed study was precluded. In some specimens in addition to true ulcer, or in the absence of an ulcer in the segment excised, distinct abnormalities were found. Lymphocytic and plasma cell infiltration exceeded normal limits and healed and healing erosions were found. No acute erosions were encountered. In some specimens the portion of the duodenum available showed no change of significance.

The frequency of abnormalities of the antral mucosa in cases with ulcer is well emphasized in Table IX, where the autopsy and surgical material may be compared. While the antral mucosa was never normal or "almost normal" among the 98 cases with ulcer, 212 of the 260 autopsy specimens were normal or almost normal. Among the 48 remaining specimens the changes were infrequently as severe as in the group with ulcer. If only the age groups of cases with ulcer are considered, the autopsy material revealed comparable degrees of gastritis in 42 of 229 cases, an incidence of 18 per cent as compared to an incidence of 100 per cent in the group with ulcer.

It may be mentioned here that attempts have been made to explain the antral findings in resected stomachs on the basis of surgical trauma. Schindler, Necheles and Gold²² reproduced the conditions incident to gastrectomy in dogs by clamping off the stomach and tying the vessels. In parts of the stomach deprived of blood supply and exposed to acid they described alterations and hemorrhages. Their illustrations are not convincing and, when correlated with the resected human stomach, show no changes of significance.

Sanders and Mecray,²³ in a similar study, described superficial hemorrhages as gastritis and concluded that when free acid was present in the stomach the changes were proportional to the degree of vascular engorgement and to the time elapsed between resection and examination of the specimen. Their illustrations showed no evidence of gastritis and they admitted the absence of leukocytic infiltration. These authors concluded that gastroscopic examination is a better means of determining the condition of the antral mucosa than is its histologic appearance in resected stomachs. Engorgement and edema admittedly

TABLE IV

Summary of Findings, as Graded, in the Body Mucosa of Cases with Duodenal Ulcer

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Extent			Group*				Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4	
11-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
21-30	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	8
31-40	8	1	0	6	0	0	0	0	0	0	0	0	0	0	0	4	5	13	2	0	9	0	24
41-50	9	0	0	4	2	0	1	0	0	0	0	0	0	0	0	5	4	10	7	1	3	0	21
51-60	5	0	0	6	0	0	0	0	0	0	0	0	0	0	0	1	4	12	5	0	0	0	17
61-70	4	0	0	3	0	0	1	0	0	1	0	0	1	0	0	0	2	2	2	2	1	1	6
Totals	27	1	0	19	3	0	2	0	0	1	0	0	1	0	0	0	13	15	46	16	2	14	78

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis; group 4 = cases with hypertrophy.

result from surgical manipulation. Such effects were frequently observed and as frequently ignored in the surgical material under consideration in this paper. It is inconceivable that atrophy, dense lymphocytic infiltration and healed erosions could have developed during the course of an operation even several hours long. Furthermore, entirely similar changes are found at autopsy in stomachs with ulcer when no operative trauma has complicated the picture.

The histologic findings in the body mucosa of the 106 cases of duodenal and gastric ulcer are summarized in Tables IV (duodenal ulcer), V (duodenal and gastric ulcer) and VI (gastric ulcer). Here again the mucosal rolls afforded a distinctly advantageous means of visualizing the general topography of the changes. With these resections in which 75 to 80 per cent of the stomach was routinely removed, many ordinary sections would have been required to provide the same information. Gastritic changes involving that portion of the body mucosa immediately adjacent to the transition zone were not considered. Only those where the process involved more than a segment of 1 to 2 cm. immediately adjacent to the antrum were considered abnormal.

Of the 78 resected specimens from cases of duodenal ulcer, the body

mucosa (Table IV) was without change in 46 instances (59 per cent). Sixteen cases (20.5 per cent) fell in group 2 (almost normal). Only 2 cases (2.5 per cent) fell in group 3. These, although they showed but mild infiltration, had small areas of atrophy. Fourteen cases (18 per cent) fell in group 4 (hypertrophic). In all of these the mucosa was over 1.5 mm. in thickness (Fig. 9). Four were otherwise free

TABLE V
Summary of Findings, as Graded, in the Body Mucosa of Cases with Duodenal and Gastric Ulcer

Age	Infiltration			Follicles			Atrophy			Meta-plasia			Pseudopyloric glands			Extent			Group*				Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4	
31-40	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0		1
41-50	1	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	1	1	2	0	1	1	4
51-60	0	3	0	0	2	0	1	0	0	0	0	0	0	0	0	1	2	1	0	3	1		5
61-70	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	3
Totals	2	5	1	3	4	0	3	0	0	0	0	0	0	0	0	1	2	5	4	1	6	2	13

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis; group 4 = cases with hypertrophy.

TABLE VI
Summary of Findings, as Graded, in the Body Mucosa of Cases with Gastric Ulcer

Age	Infiltration			Follicles			Atrophy			Meta-plasia			Pseudopyloric glands			Extent			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
31-40	1	1	1	1	2	0	1	0	1	0	0	1	1	1	0	0	1	2	0	1	2	3
41-50	1	3	0	3	1	0	2	1	0	2	0	0	2	1	0	1	1	2	0	1	3	4
51-60	1	5	0	4	2	0	2	3	0	3	0	0	3	1	0	0	4	2	0	1	5	6
61-70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71-80	0	2	0	2	0	0	0	1	1	2	0	0	2	0	0	0	1	1	0	0	2	2
Totals	3	11	1	10	5	0	5	5	2	7	0	1	8	3	0	1	7	7	0	3	12	15

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis

of change. Abnormalities exhibited by the remaining 10 consisted of superficial lymphocytic and plasma cell infiltration only; mild in 9, moderate in 1. In the table these features are included with those of the nonhypertrophic specimens. If the entire group is considered from the standpoint of the gastritic features only, 50 (64.1 per cent) were free of change, 25 (32 per cent) showed mild superficial lymphocytic infiltration only, 1 (1.3 per cent) showed moderate superficial infiltration only and 2 (2.6 per cent) showed in addition small areas of atrophy. The extent of the involvement was partial in 13 and total in 15.

The findings in the 13 cases with both duodenal and gastric ulcers are summarized in Table V. Four were normal, 1 was almost normal, 6 were gastritic and 2 were hypertrophic. Of the hypertrophic

specimens 1 was otherwise unchanged and 1 showed mild superficial lymphocytic infiltration.

The findings in the cases of gastric ulcer are summarized in Table VI. Among these none were normal, 3 were almost normal and 12 showed gastritis. All 12 showed at least some degree of atrophy.

Because of the differences in the findings in the three groups of cases with ulcer the data have not been consolidated. Each group may best be considered in relation to the autopsy material (Table X). Excluding instances of hypertrophy the character of the changes found in cases with ulcer and without ulcer is the same.

Those specimens in the group with duodenal ulcer in which the mucosa was abnormal showed the same age distribution as did those of the autopsy series. The total number of cases per decade was small and the numbers in the two groups were not strictly comparable. However, there appeared to be a greater incidence of mild infiltration in the group with ulcer for each decade concerned. On the other hand, severe alterations—atrophic mucosae—were much less frequently seen. In the group with ulcer there were 44 cases in the fifth to seventh decades inclusive and only 2 of these showed atrophic changes, both mild. Among the 100 autopsy cases for the same period there were 23 instances of atrophic gastritis. The distribution of the changes could not, of course, be compared. It may be reasonably concluded that in spite of the constant finding of an antral gastritis in cases of duodenal ulcer, severe changes in the body mucosa only exceptionally appear and are less numerous than might be expected in an equivalent number of individuals free of ulcer in the older age groups.

Cases in which both gastric and duodenal ulcers were present were intermediate in position between the groups with duodenal and with gastric ulcer as to changes in the body mucosa. The data may be compared with those from autopsy material in Table X, but the small number of cases precludes any conclusions.

In the group with gastric ulcer there were no specimens in which the body mucosa was entirely without change and 12 of the 15 showed at least some areas of atrophy. The cases were rather evenly distributed through the several decades and the incidence of atrophy was high in each. That atrophic changes are to be expected in cases of gastric ulcer is suggested. However, with so few cases the remote possibility of a coincidental high incidence cannot be excluded.

In this group of 106 cases with ulcer the changes were independent of all considerations other than their association with ulcer. The great majority of these patients came to operation only after years of medical management which had given no constant relief. A few had

had previous gastroenterostomies and some had had one or more hemorrhages. Many of them had high fasting acids and copious secretion. Many of them had, or at some time had exhibited, symptoms or roentgenologic evidence of obstruction. As far as the antral changes were concerned there was no relation to the character or duration of symptoms or to the exhibited secretory activity. The mucosal changes were as severe in a boy of 13 years whose symptoms covered a 3-year period as they were in much older individuals who reported periodic episodes of distress of 10 or even 20 years' duration. Patients in whom the body mucosa was found to be hypertrophic showed no greater acidity or more severe symptoms than did those in whom the mucosa was of normal thickness. Changes in neither the antrum nor the body were in any way related to obstruction. The body mucosa was found to be entirely normal in cases of long-standing, high-grade obstruction, while in others, in which no obstruction had occurred, there was a superficial gastritis. The cases of gastric ulcer in general showed lower acidity than did the group with duodenal ulcer. Perhaps this finding is related to the higher incidence of atrophic gastritis in the cases of gastric ulcer, although the case which presented the most extensive atrophic changes had normal acid values. There was no relationship between the findings and the size of the ulcer, or the presence or absence of perforation. In some cases very small ulcers were found which were hardly consistent with the duration of symptoms. The cases in which the resected specimens showed acute erosions and those with a grossly demonstrable erosive gastritis had no unusual clinical features.

DISCUSSION OF GASTRITIS ASSOCIATED WITH ULCER

The findings in this series of cases support the frequently reported constancy of a gastritis associated with ulcer. There is good evidence that the process is primary and not secondary to the ulcer. The frequent antrum-wide distribution is by itself evidence for its primary character. Additional evidence is also to be gained from the not infrequent instances in which stomachs resected for typical symptoms of ulcer have failed to reveal ulcers but have shown an erosive gastritis and duodenitis (Puhl,¹⁴ Aschner and Grossman,¹⁷ Konjetzny,²⁴ Faber,²⁵ Paaby,²⁶ Nicholaysen,²⁷ Dahl,²⁸ Holsti,²⁹ Roholm³⁰ and Andersen³¹).

It appears, then, that true ulcer is superimposed on the gastritis (and duodenitis) to the erosive lesions of which Konjetzny,¹ Puhl¹⁴ and others traced its origin. In the chronic changes they saw the healed and healing stages of repeated acute inflammatory attacks. Puhl considered that periodicity of symptoms presented by patients with

ulcer found anatomic expression in the simultaneous presence of acute erosions and their healing stages in resected specimens. Further evidence for this concept was provided by the stomachs of calves in which the appearance of ulcers following weaning had been long known. In these animals Konjetzny and Puhl³² found acute and chronic inflammatory changes and all transitions from erosions to true ulcers. In my own human material the changes are so predominantly chronic that an evolution from acute inflammatory attacks cannot be traced. It has been suggested that longer and more adequate preoperative medical management accounts for the infrequency of acute lesions in the more recent material (Aschner and Grossman¹⁷). Puhl's¹⁴ case histories, for example, reveal that operative interference was employed at the height of acute symptoms in many instances.

Experimental evidence for a gastritic basis for chronic ulcer is seen in the cinchophen ulcers produced in dogs (Van Wagoner and Churchill,³³ Bollman, Stalker and Mann³⁴ and Simonds³⁵) and cats (Schwartz and Simonds³⁶) where an acute diffuse gastritis with multiple erosions precedes the larger lesion.

Other recent experimental work has emphasized the importance of the acid factor in ulcer genesis. Although numbers of investigators have reported superficial erosions as the result of elevated gastric secretion in animals (Büchner, Siebert and Molloy,³⁷ Simpson,³⁸ Overgaard³⁹ and others), no lesions similar to the chronic ulcer of man were produced. Employing Code and Varco's⁴⁰ method of daily injections of histamine-base in beeswax for inducing prolonged hypersecretion, Walpole⁴¹ and Hay⁴² with their collaborators have produced true chronic ulcers in cats, dogs and other animals. The changes preceding the appearance of the ulcers have not been described. Some of their specimens which I have been privileged to examine showed no significant mucosal alterations near to, or at a distance from, the ulcers. If anything resembling a diffuse gastritis preceded the appearance of actual ulceration, it left no traces. Whether such changes may have occurred or whether the acidity attained in these animals resulted in a primary cauterization of the mucosa is not known.

While acid must be admitted as a constant factor in the problem of "peptic" ulcer, the antral gastritis is also constant. Its predominant limitation to the antrum indicates that it is a special form of gastritis which must be separated from the process leading to atrophy and achlorhydria where the body is regularly involved. If acid is to be considered the prime factor in the causation of ulcer, it must also be responsible for the accompanying gastritis. That acid secretion anywhere within possible physiological limits can initiate such a process

seems doubtful. While ulcer patients often secrete copious amounts of highly acid gastric juice, whether such secretory activity prevailed prior to the development of ulceration is not known. Faber² considered this hypersecretion to be largely accounted for by the pathologic aftersecretion resulting from the irritating antral gastritis. While it is not theoretically impossible that acid may cause or be a factor in the causation of a gastroduodenitis, the gaps between the condition in man and the acid production of ulcer in animals are wide. That acid produces the constantly observed changes in the antral mucosa requires demonstration.

No conclusive evidence pointing to an etiologic factor for the gastritis with ulcer has been produced. Konjetzny¹ emphasized the importance of a variety of exogenous irritants. In this connection he attributed the gastritis of calves to the coarse foods which supplanted milk at the time of weaning. That certain foods may under certain circumstances have a deleterious effect has been suggested by experimental observations. Bollman, Stalker and Mann³⁴ found that coarse foods decreased and soft foods increased the time required for the appearance of cinchophen ulcers in dogs. Simpson³⁸ was able to produce erosions in his animals with sustained high acid values only when mustard oil was added to their food. However, the importance of food irritants as a factor in the production of this form of gastritis in man is as difficult to substantiate as it is to consider them of importance in the evolution of atrophic pangastritis.

Irrespective of the primary basis of the antral gastritis there is no doubt that the changes constantly accompany gastric ulcer, and Konjetzny's²⁴ original dictum that an ulcer does not develop in a healthy mucosa still holds for the majority of the cases.

SIMPLE GASTRITIS IN RELATION TO CARCINOMA OF THE STOMACH

The frequent association of widespread gastritis and carcinoma of the stomach has long been known, and has been discussed by many authors on the basis of both autopsy and surgical material (Geissendörfer,⁷ Borchardt,¹⁵ Puchert,¹⁶ Simpson,¹⁸ Mathieu,⁴³ Saltzman,⁴⁴ Konjetzny,^{1, 45, 46} Orator,⁴⁷ Judd⁴⁸ and others). The diffuse distribution of these mucosal alterations rather sharply distinguishes the process from that associated with ulcer. Several authors have commented upon the differences. Konjetzny,¹ Orator,¹³ Borchardt¹⁵ and others have emphasized the fact that the gastritis of ulcer is antrum-confined for the most part while that of cancer is a pangastritis. Although this point remains the chief distinguishing feature, other

dissimilarities have been noted. Intestinal metaplasia is largely focal with ulcer and often diffuse with cancer (Geissendörfer,⁷ Moszkowicz,⁹ Kalima,¹² Borchardt¹⁵). Larger numbers of Russell's corpuscles are seen in stomachs with cancer than in those with ulcer (Konjetzny,¹ Kalima,¹² Puchert¹⁶). Konjetzny¹ claimed that the gastritis associated with ulcer showed more evidence of hyperplasia and less outspoken atrophy. Largely on this basis Konjetzny considered the gastritis with ulcer as a subacute process in contrast to the chronic gastritis with carcinoma. All of these changes are, however, subject to variation and no constant distinctions exist for the single case.

Of particular interest in the matter of gastritis associated with carcinoma is the question of its relationship to the tumor. Most of the early writers considered the gastritis to be independent of, or in some way secondary to, the tumor (Rosenheim,⁴⁹ Matti⁵⁰). Although Mathieu⁴³ had early suggested the origin of carcinoma on the basis of a pre-existing gastritic mucosa, it was not until Saltzman⁴⁴ and Konjetzny⁴⁵ independently presented their material that the gastritic basis for carcinoma was seriously considered. Konjetzny^{1, 45, 46} saw in the abnormal reparative processes of gastritis, particularly adenomatous polypoid overgrowth, all transitions to frank carcinoma. He claimed a demonstrable relationship to the parenchymal changes of chronic gastritis for 90 per cent of carcinomas, and further pointed out that the early carcinomas described by Hauser, Versé and others presented a similar relationship. That a carcinoma develops more commonly in stomachs which show chronic atrophic gastritis has come to be rather generally accepted (Orator,¹³ Hurst⁵¹). Several authors have commented upon the coincidence of pernicious anemia and carcinoma of the stomach and have maintained that atrophic gastritis is the common link (Miller,⁵² Jenner⁵³ and others).

There have been objections to the concept established by Saltzman⁴⁴ and Konjetzny.⁴⁵ Borrmann,⁵⁴ in particular, questioned the interpretation of the histologic findings of those authors, and doubted that the changes they described necessarily preceded the carcinoma. He believed that the "gastritis to gastritis polyposa to carcinoma" sequence stressed by Konjetzny^{45, 46} was very much the exception, and concluded that most carcinomas arose without, or independent of, pre-existing inflammatory changes. Wanser⁵⁵ rightfully emphasized the benign character of the frequently long-standing chronic gastritis associated with ulcer and doubted the existence of a causal relationship between such mucosal changes and carcinoma.

The mucosal findings in the resected specimens from 52 cases of carcinoma of the stomach are reported here. Only those specimens

which afforded relatively large amounts of mucosa uninvolved by tumor were included. Because the tumor most frequently occupied the antrum, the findings in the antral mucosa were recorded in but 27 cases. Similar circumstances excluded the body in 2 cases.

The antral findings are summarized in Table VII. There were no cases in which the antral mucosa was normal although in most the changes were relatively mild. In Table IX the changes may be compared as to severity with those associated with ulcer and in material obtained postmortem.

TABLE VII

Summary of Findings, as Graded, in the Antral Mucosa of Cases with Carcinoma of the Stomach

Age	Infiltration			Follicles			Atrophy			Metaplasia			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
31-40	1	1	0	2	0	0	1	1	0	0	1	0	0	0	2	2
41-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51-60	5	2	0	5	0	0	5	0	1	2	0	1	0	1	6	7
61-70	9	4	0	7	2	0	6	2	3	4	2	3	0	2	11	13
71-80	3	2	0	3	1	0	4	0	1	1	1	1	0	0	5	5
Totals	18	9	0	17	3	0	16	3	5	7	4	5	0	3	24	27

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

TABLE VIII

Summary of Findings, as Graded, in the Body Mucosa of Cases with Carcinoma of the Stomach

Age	Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Extent			Group*			Total
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
31-40	0	2	0	2	0	0	1	1	0	1	0	0	2	0	0	0	0	1	0	0	2	2
41-50	0	3	0	2	1	0	0	0	3	1	1	0	0	3	0	0	0	3	0	0	3	3
51-60	5	6	1	8	2	0	0	0	3	9	1	7	7	2	2	1	2	0	1	0	12	13
61-70	5	10	3	11	5	0	0	2	13	4	4	6	7	6	2	2	6	10	2	2	16	20
71-80	7	4	0	6	1	0	0	3	6	1	1	4	6	3	0	3	2	6	1	2	9	12
Totals	17	25	4	29	9	0	1	9	31	10	7	17	22	14	4	6	11	29	4	4	42	50

* Group 1 = normal cases; group 2 = almost normal cases; group 3 = cases with gastritis.

The findings in the body mucosa are summarized in Table VIII. Four were normal, 4 almost normal and 42 distinctly abnormal. Of the 42, 41 showed atrophic changes in at least some degree and extent. These findings may be compared with those of the ulcer and autopsy groups in Table X. In this table the cancer cases showing gastritis with or without atrophy appear in one column and those with atrophy in another.

DISCUSSION OF GASTRITIS ASSOCIATED WITH CARCINOMA

That gastritis associated with carcinoma is a pangastritis in contrast to the antral gastritis of stomachs with ulcer has been noted. There

is no direct evidence as to the time required for the development of an atrophic gastritis, but if carcinomas are prone to develop in stomachs which show an atrophic gastritis such as is commonly seen in pernicious anemia, the changes should, in the majority of cases, be extensive and severe. It is of interest to compare the mucosal changes in this series of stomachs with carcinoma with those found in a group of noncancerous stomachs in which atrophic gastritis was present. For this purpose 7 resected stomachs were available, some of which showed benign polyps and others only an atrophic gastritis.

TABLE IX

Findings in Antral Mucosa in Control Autopsy Material and in Cases of Ulcer and Cancer of the Stomach

Age	Autopsy material				With ulcer				With carcinoma			
	Total	Normal	Almost normal	Gastritis	Total	Normal	Almost normal	Gastritis	Total	Normal	Almost normal	Gastritis
Under 1	16	16	0	0
1-10	27	26	0	1
11-20	11	6	4	1	2	0	0	2
21-30	24	19	3	2	8	0	0	8
31-40	47	31	9	7	26	0	0	26	2	0	0	2
41-50	27	17	7	3	26	0	0	26	0	0	0	0
51-60	36	17	9	10	27	0	0	27	7	0	1	6
61-70	27	15	11	11	7	0	0	7	13	0	2	11
71-80	27	13	6	8	2	0	0	2	5	0	0	5
81-90	8	2	1	5
Totals	260	162	50	48	98	0	0	98	27	0	3	24

The resections were as high as in the other group so the mucosa could be examined at equivalent levels. The antral changes were of the same character as those found in the carcinoma group. While the number of cases was small, the changes appeared to be of the same magnitude. The body mucosa was severely affected in all 7 cases. Each specimen showed an advanced atrophic gastritis in which very few if any specific glands remained. These findings are comparable to those in many of the cancer group and are strikingly similar to cases where the carcinoma developed in individuals with pernicious anemia. The similarity is much less marked in other cases. Referring to Table VIII, the extent of involvement in the 50 body mucosas was: none in 4 cases, grade 1 in 6 cases, grade 2 in 11 cases and grade 3 in 29 cases. In those where extent was graded 1 and 2, that portion of the mucosa adjacent to the tumor—the lower body for the most part—showed changes, while the upper one-half or one-third of the segment was free of significant change. Thus there were all transitions, from mucosas completely normal to those where the whole segment

TABLE X
Findings in Body Mucosa in Material Obtained by Autopsy and in Cases of Ulcer and of Cancer of the Stomach

Age	Autopsy material					With duodenal ulcer					With duodenal and gastric ulcer					With gastric ulcer					With carcinoma						
	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy	Total number	Normal	Almost normal	Total gastritis	Gastritis with atrophy		
Under 1	16	16	0	0	0	78	46	16	2	2	14	13	4	1	6	3	2	15	0	3	12	12	50	4	4	42	41
1-10	27	25	1	1	0	2	7	0	0	0	0	1	0	0	0	0	0	0	0	0	3	3	0	0	3	3	2
11-20	11	9	2	0	0	8	24	13	0	0	1	4	2	0	0	0	0	3	0	1	3	3	3	0	0	3	3
21-30	24	19	4	1	0	2	10	7	0	0	0	5	1	0	1	1	1	6	0	1	5	5	13	1	0	12	12
31-40	47	30	9	8	2	21	12	5	0	1	3	5	1	0	3	1	0	4	0	1	5	5	20	2	2	16	15
41-50	27	21	3	3	1	6	17	2	1	1	1	3	1	0	1	1	0	2	0	0	2	2	12	1	0	9	9
51-60	36	17	3	16	12	11	11	2	1	1	1	3	1	1	1	1	0	2	0	0	2	2	12	1	0	9	9
61-70	37	16	6	15	6	9	17	2	1	1	1	3	1	1	1	1	0	2	0	0	2	2	12	1	0	9	9
71-80	27	17	2	8	4	3	17	2	1	1	1	3	1	1	1	1	0	2	0	0	2	2	12	1	0	9	9
81-90	8	3	0	5	4	3	17	2	1	1	1	3	1	1	1	1	0	2	0	0	2	2	12	1	0	9	9
Totals	260	173	30	57	35	78	46	16	2	2	14	13	4	1	6	3	2	15	0	3	12	12	50	4	4	42	41

was abnormal. Moreover, variable degrees of extent as to severity of the process existed within the group graded 3. As already indicated, many of these specimens showed changes identical with those found in the noncancerous stomachs. In an appreciable number, however, the following appearances were presented:

a. Lower body adjacent to the tumor: Thin mucosa exhibiting complete loss of specific glands, more or less metaplasia to an intestinal type and variable lymphocytic infiltration.

b. Midportion of segment: Mucosa of normal thickness, some specific glands retained, while others showed deepened, regularly arranged pits lined in part by intestinal epithelium, some pseudopyloric glands and variable degrees of lymphocytic infiltration.

c. In upper part of segment: Mucosa of normal thickness with mild to moderate superficial lymphocytic infiltration but glands retained. An appearance such as that just described was by no means as sharply defined as this outline indicates, but that general pattern was presented and even in those cases where destruction and disorganization of the mucosa involved the higher reaches of the segment the process was frequently less pronounced near the plane of excision.

In one specimen in which the tumor was situated at the cardia, the changes presented themselves in the reverse manner. The most pronounced gastritic alterations were at the upper end of a segment from the greater curvature, and the mucosa became almost normal as the antrum was approached.

The apparent manner of development and progression to atrophy of the process just described and the presence in this series of several completely normal and other only partially involved body mucosas indicated that carcinoma in a distinctly appreciable number of cases was not preceded by a diffuse atrophic gastritis. In general, the most severe atrophy was associated with the larger tumors. Yet of the 4 cases in which the body mucosa was normal, 3 had relatively large antral tumors. There was no correlation between the degree of atrophic gastritis and the severity or duration of obstruction. There was no evidence that any carcinoma in this series had developed on the basis of a pre-existing ulcer. (Some authors have indicated that in stomachs with cancer but without diffuse gastritis the carcinoma is a malignant change in an ulcer: Borchardt,¹⁵ Puchert,¹⁶ Orator,⁴⁷).

That the atrophic process may develop under the influence of the tumor is suggested by the findings detailed above. Particularly is that true in the case of the tumor at the cardia, where, contrary to the usual pattern of an ascending atrophy, the atrophy proceeded from above downward. Yet one cannot eliminate the possibility that some

of these cases are examples of the coincidental relationship of a developing atrophic gastritis, which is not uncommon in the age periods concerned, and a carcinoma. This explanation is plausible, but in the absence of known etiologic factors for an atrophic gastritis, no definite conclusions are justified.

That an appreciable number of these carcinomas apparently developed in stomachs which were not the seat of a long-standing atrophic gastritis affects but little the question of the primary genesis of the tumor. The tumors were, for the most part, large and beyond the stage at which evidence for their early development could be found. It is true that, particularly among the tumors associated with a pre-existing diffuse atrophic gastritis, there was often at the growing margins of the tumor a blending of the carcinoma with the abnormal glands. In some specimens there were multiple foci of origin in an atrophic, metaplastic mucosa beyond the main body of the tumor. In a few specimens there were transitions between polypoid overgrowths and carcinoma. In other instances the mucosa in the immediate vicinity of relatively large carcinomas showed little change. That finding alone cannot eliminate the possibility that the tumor concerned arose in a localized abnormal area in the mucosa. One may agree with Borrmann⁵⁴ that carcinomas developing on the basis of a gastritis polyposa are the exception rather than the rule. The earliest carcinomas in this series were flat, ulcerating lesions which, on a somewhat larger scale, followed the ulcerative changes noted by Mallory⁵⁵ in his cases of carcinoma *in situ*.

SUMMARY AND CONCLUSIONS

The manifestations of chronic gastritis encountered in 260 stomachs obtained at autopsy, in 106 stomachs resected for duodenal or gastric ulcer and in 52 stomachs resected for carcinoma are reported.

The autopsy series included individuals of all ages, dying from a variety of causes, whose histories revealed no evidence of gastric symptoms. The changes exhibited by this group were chiefly those of a pangastritis leading to atrophy. The process bore no demonstrable relationship to any factor other than age. Gastritic changes in any degree were rare below the age of 30 years and severe changes were uncommon below the age of 50. Atrophic gastritis was exhibited by 30 per cent of the 108 cases past 50 years of age. This form of gastritis is unimportant in resected stomachs from individuals under 50 years of age.

Among the 106 stomachs resected for ulcer (78 duodenal, 13 gastric and duodenal, 15 gastric) there was an antral gastritis in all of the

98 cases in which the antrum was examined. Changes in the body mucosa were rare in the duodenal ulcer group and common in the gastric ulcer group. The evidence substantiates the contention that an antral gastritis (and duodenitis) precedes, and is the anatomic basis for, the development of chronic ulcer.

The gastritic changes encountered in 52 resected carcinoma-bearing stomachs were variable in extent and severity. Although numbers of these stomachs presented a diffuse atrophic gastritis which undoubtedly antedated the tumors, that condition did not obtain for all. In a few instances the body mucosa was normal or nearly normal. In others, although there were severe changes in the vicinity of the tumor, the more distant portions of the excised segments were less, or not at all, affected. The findings in such cases suggested that the mucosal changes were secondary to the tumor, although the possibility of a coincidental association of the tumor and a developing atrophic gastritis could not be excluded. There was in this series no evidence to indicate that carcinomas arise with unusual frequency in stomachs already the seat of a diffuse atrophic gastritis.

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DESCRIPTION OF PLATES

PLATE 7

- FIG. 1. Normal body mucosa. Hematoxylin and eosin stain. $\times 75$.
- FIG. 2. Body mucosa showing advanced atrophy, cystic and pseudopyloric glands and moderate lymphocytic infiltration. Hematoxylin and eosin stain. $\times 75$.
- FIG. 3. Body mucosa showing mild superficial lymphocytic infiltration. The glands are intact. Hematoxylin and eosin stain. $\times 75$.
- FIG. 4. Body mucosa showing moderate lymphocytic infiltration and moderate atrophy. There are deepened pits. The basilar portions of the glands are well retained. Hematoxylin and eosin stain. $\times 75$.

65451

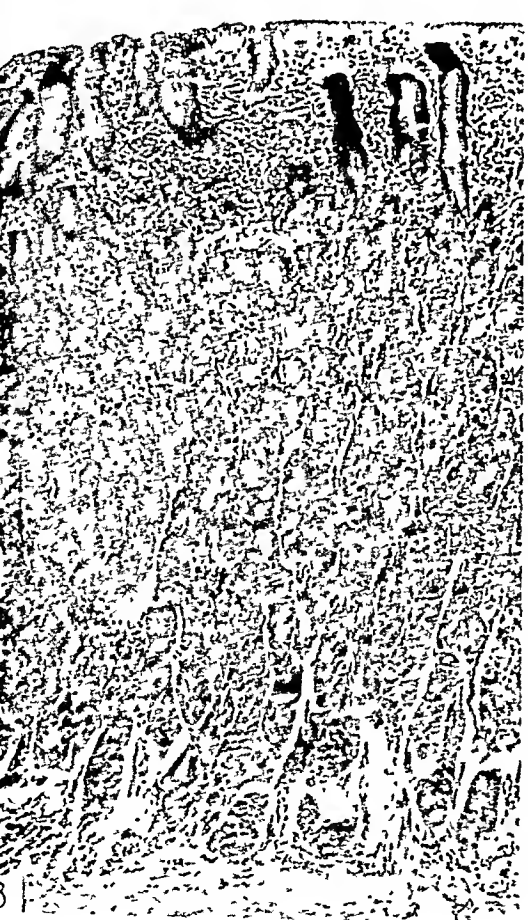
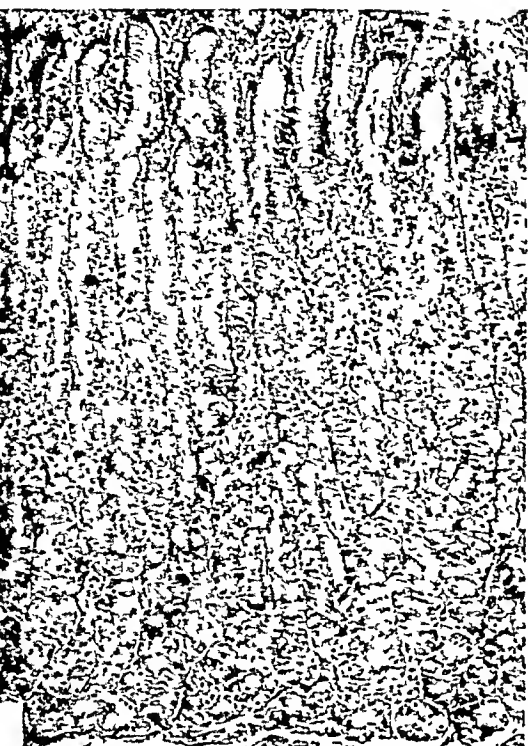


PLATE 8

FIG. 5. Normal antral mucosa. Hematoxylin and eosin stain. $\times 75$.

FIG. 6. Antral mucosa showing mild lymphocytic infiltration and a single lymphoid follicle. The glands are intact. Hematoxylin and eosin stain. $\times 75$.

FIG. 7. Antral mucosa showing advanced atrophy, heavy lymphocytic infiltration and follicles. Hematoxylin and eosin stain. $\times 75$.

FIG. 8. Focal area of metaplasia to the intestinal type in an atrophic body mucosa. Hematoxylin and eosin stain. $\times 75$.



Hebbel

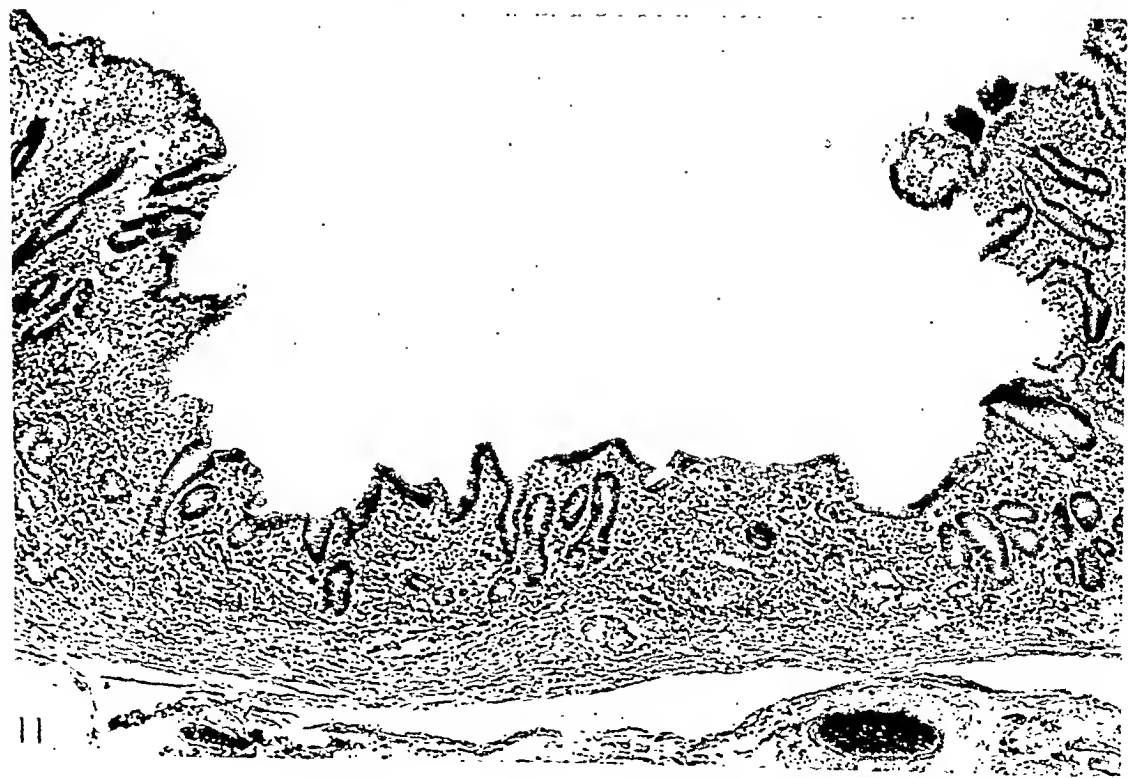
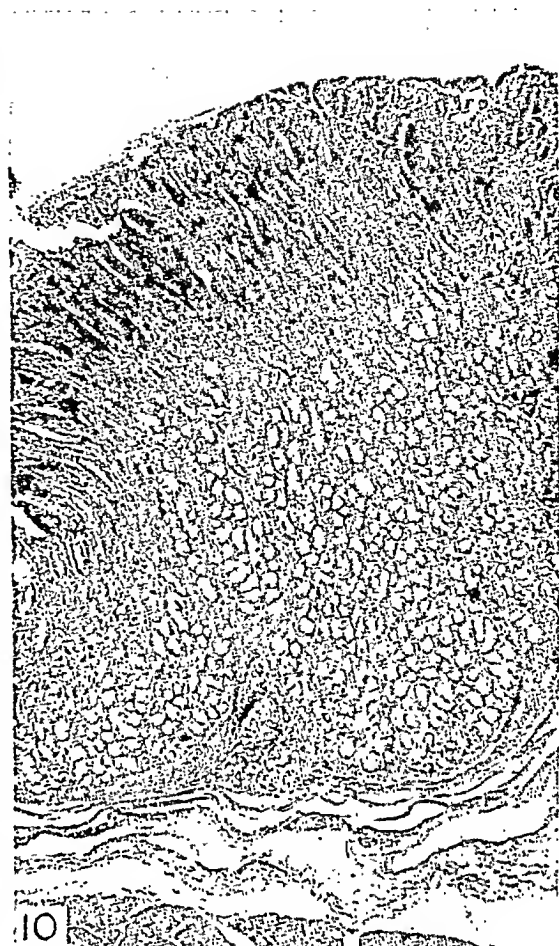
Chronic Gastritis

PLATE 9

FIG. 9. Hypertrophic body mucosa. Hematoxylin and eosin stain. $\times 30$.

FIG. 10. Hypertrophic antral mucosa. Hematoxylin and eosin stain. $\times 30$.

FIG. 11. Healed erosion in antral mucosa. Hematoxylin and eosin stain. $\times 75$.



THE LYMPH NODES IN DISSEMINATED LUPUS ERYTHEMATOSUS *

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INTRODUCTION

Enlargement of the superficial and deep lymph nodes, a prominent finding in the majority of reported cases of disseminated lupus erythematosus, has heretofore received only brief mention. In the early literature lymphadenopathy was considered to be pathognomonic, *per se*, of tuberculous infection, and much effort was expended to prove that the "systemic" form of lupus erythematosus was a manifestation of generalized tuberculosis. But in more recent years, largely due to the investigations of Keil (1937) and others, this contention has been found to be untenable.

In 1872, Kaposi reported a group of cases which, he believed, presented a syndrome separate from that of the already well known "discoïd type" of lupus erythematosus. He remarked that "in several of the acute cases generalized hard painful swelling of the lymph nodes, especially cervical, axillary and occasionally inguinal" was noted; but in the detailed case reports he mentioned enlarged cervical nodes in only 2 cases, and enlarged cervical and axillary nodes in 1 other case. Only 2 of his 11 cases came to autopsy.

Hardaway (1889), in reporting an example of this disease 17 years after Kaposi's original description, noted "great swelling of the lymphatic glands in the front of the neck." In 1892 he reported another case in a girl of 14 years, and stressed the fact that the "submaxillary glands were swollen somewhat but not excessively."

Although the general impression at the turn of the century was that disseminated lupus erythematosus was caused by the tubercle bacillus, examination of the evidence presented fails to exclude the coincidental occurrence of tuberculosis, a common disease at the time, in an already debilitated patient. Keil (1937) concluded "that the occurrence of tuberculosis in cases of lupus erythematosus is coincidental and unrelated." Sequeira and Balean (1902) found "that the disseminated form of lupus erythematosus is associated with tuberculosis to a much greater degree than the discoïd variety," yet the single case which they reported had no evidence of tuberculous infection whatsoever.

Roberts (1911), in reporting a case of disseminated lupus erythematosus, was so impressed by the relationship between the lymphad-

* Received for publication, May 29, 1942.

enopathy and the disease entity that he postulated a theory to explain how this association was affected:

"The relationship [of tuberculosis to lupus erythematosus disseminatus] probably only comes into play when certain organs of the body are attacked by the tubercle bacilli. The researches of Boeck seem to indicate that these special organs are the lymphatic glands. . . . Now, while we cannot admit that lupus erythematosus is a manifestation of tuberculosis or tuberculin poisoning, we are met by the fact that the disease is more frequently associated with adenitis than with any other morbid antecedent, and that this adenitis is very often of a tubercular nature. . . . It is at least conceivable that the cytolytic toxin of lupus erythematosus is derived from the leucocytes in the lymph-glands, and that the formation of the toxin, although not absolutely dependent on the presence of tubercle bacilli, is favored by the presence of the bacillus in the gland. . . . From the first, some depressant influence is at work tending towards lymph stagnation, and it is under this condition that I conceive the origin of the cytolytic poison in, or by, the leucocytes of the lymph-glands."

It is interesting to observe that when the reported cases were reviewed chronologically, the incidence of tuberculous lymphadenitis associated with disseminated lupus erythematosus diminished, although the search, especially microscopically, for tubercle bacilli and tuberculous lesions became more diligent. Scrutiny of the detailed accounts of the early cases of the disease fails to divulge convincing evidence on which to base the concept of these cases as tuberculous. Low and Rutherford (1920) described enlarged mediastinal, para-aortic, mesenteric and cervical nodes in their case, and stated emphatically that evidence of tuberculosis was not found. Ehrmann and Falkenstein reported 6 cases of this disease in 1922; 5 patients had lymphadenopathy, and in only 2 of these was there evidence of tuberculosis; however, even in the 2 positive cases only "some nodes showed tuberculosis, others not." In 1 case, injection of portions of a "tuberculous node" into a guinea pig failed to produce evidence of tuberculosis. Keith and Rowntree, in the same year, reported 4 cases, in all of which there were enlarged lymph nodes. Only 1 case came to necropsy and in this miliary tubercles were found in the spleen. Examination by biopsy of a cervical lymph node in another case revealed tuberculosis. Gennerich (1921), Sibley and Wynn (1923), Gibson (1925) and Clarke and Warnock (1926) were unable to demonstrate evidence of tuberculosis in their cases. Keefer and Felty (1924) described tuberculous lymphadenitis in 2 of their 3 cases, although all had generalized lymphadenopathy. Lyon (1933) failed to produce tuberculous lesions in a guinea pig with portions of enlarged hepatic, cervical and tracheobronchial nodes from 1 of his 2 cases. Baehr, Klemperer and Schiffrin (1935) found that "evidences of active tuberculosis were absent in all but two" of their 23 cases. "One had a

single caseous tracheobronchial lymph node. Another had terminal acute miliary tuberculosis." Rose and Pillsbury, writing in 1939, agreed with Keil that "proof of the tuberculous etiology in disseminated lupus erythematosus is lacking, and that the coincidence of active tuberculosis is not necessarily significant." None of our 3 cases (to be described in detail) showed any evidence of tuberculosis in spite of diligent search for specific lesions.

We shall subsequently attempt to show that not only is the absence of tuberculous lymphadenitis the rule, but the morphologic characteristics of the enlarged nodes, independent of blood vessel alterations, are more or less constant and perhaps peculiar to the disease. With

TABLE I

Age, Sex and Color Distribution of 280 Cases of Disseminated Lupus Erythematosus

Age in years	0-10	11-20	21-30	31-40	41-50	51-60	61-70	Age not stated	Total
Females	7	53	73*	39	20	6	4	23	225
Males	2	15	14	7	8	1	0	1*	48
Total	9	68	87	46	28	7	4	24	273†

* One Puerto Rican.

† All patients included were white. Not included are 7 cases: 1 male Hawaiian of Japanese extraction, 25 years of age; 3 Negroes, 24, 35 and 38 years of age, and 3 white patients whose age and sex were not given.

this thought in mind we reviewed most of the reported cases of disseminated lupus erythematosus in an effort to determine the incidence of lymph node enlargement as part of the morbid anatomy, and to gather the gross and microscopic impressions of other observers concerning this alteration.

In our review we paid special attention to lymph node enlargement, either clinically or at necropsy, and to findings which we believe are pertinent to this particular aspect of the disease; namely, splenomegaly, leukocytosis or leukopenia, and morphology of the bone marrow. Our observations, segregated according to age and sex, are tabulated in Table I. We were able to find a total of 277 reported cases, plus the 3 cases to be described. These represented over 250 different cases, after allowing for duplication of cases mentioned by different authors. There were 228 females and 49 males (in 3 cases the sex was not stated). It is significant that 127, or 55.7 per cent, of the females, and 30, or 61.2 per cent of the males, were between the ages of 11 and 30 years, a total of 157, or 62.1 per cent. (In 27 cases the age was not stated.) These figures verify the general impression that the disseminated form of lupus erythematosus is a disease

of adolescents and young adults. There were 214 cases, or 84.6 per cent, in which the patients were under 40 years of age. The youngest case was that of a male infant, 11 months of age, and the oldest a woman of 70 years. Although the disease is one which afflicts the white race predominately, without respect for nationality, and particularly individuals with fair skin, a few cases in Negroes and 1 in an Hawaiian of Japanese extraction were reported.

THE LYMPH NODES

General Remarks

The first histologic description of the enlarged lymph nodes in disseminated lupus erythematosus¹ was provided by Short in 1907. He observed that "the sinuses are filled with fibrin and large vacuolated endothelial cells. The lymphoid cells are, to a great extent, replaced by large cells with a considerable amount of protoplasm, the nuclei of which are large and oval, often duplicated, and frequently showing mitotic figures. There are areas of haemorrhage and other areas of complete necrosis."

Low and Rutherford (1920) were impressed by the "separation of the lymphoid elements" in their case "as if from edema"; there was "marked acute hyperemia, with a few minute hemorrhages into the gland substance and much endothelial cell reaction." Denzer and Blumenthal (1937) found changes compatible with acute lymphadenitis in the mesenteric nodes of their case. Madden (1932) remarked that "superficial lymphadenitis is often to be noted, especially in those regions that drain the involved skin. The inflammation in the glands does not go on to suppuration, and recedes when regression of the skin lesions takes place." Ginzler and Fox, as late as 1940, were the first to describe the lymph node alterations in great detail. They remarked that "some lymph nodes showed merely edema, sinus hyperplasia, and an inflammatory reaction manifested by the presence of many histiocytic cells, and a few plasma and polymorphonuclear cells." Many of the nodes in their case "contained numerous areas of necrobiosis, apparently in different stages of development," a finding which was absent from the nodes in our 3 cases. They divided the necrobiotic process into several stages, the earliest consisting of "foci of cellular necrobiosis with virtually no reactive inflammation. These progressed to larger areas of necrosis with complete cell degeneration and disintegration and nuclear pyknosis and karyorrhexis. Nuclear particles were spread throughout the surrounding pulp, which showed marked histiocytic proliferation." Other authors—Short (1907), Keil (1940) and Klemperer, Pollack and Baehr (1941), to

name a few,—also mentioned the areas of necrosis, so we are forced to conclude that this change, although not a consistent one, occurs frequently. Ginzler and Fox (1940) felt that "the occurrence of the focal necrotic lesions in the lymph nodes appeared to be as characteristic as the glomerular changes, though undoubtedly of less frequent occurrence." Keil (1937) was of the opinion that the areas of necrosis so frequently observed in the lymph nodes were "formerly used as proof of tuberculous source," which perhaps explain why the association of tuberculosis with disseminated lupus erythematosus was reported so often in the early literature on the subject.

TABLE II
State of Lymph Nodes in 280 Cases of Disseminated Lupus Erythematosus
Segregated According to Age and Sex*

Age in years	Enlarged			Not enlarged			Condition not stated		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
0-10	5	2	7	2	0	2	0	0	0
11-20	35	11	46	15	2	17	3	2	5
21-30	37	10	47	17	5	22	19	1	20
31-40	21	5	26	13	1	14	7	1	8
41-50	7	4	11	6	4	10	7	0	7
51-60	3	0	3	1	1	2	2	0	2
61-70	0	0	0	2	0	2	2	0	2
Age not stated	0	0	0	1	0	1	22	1	23*
Total	108	32	140	57	13	70	62	5	67*

* Three cases (age and sex not given) not included in table.

Moderate or marked enlargement of the lymph nodes in disseminated lupus erythematosus occurs too often to be considered a mere coincidental finding. The enlargement may be localized, or generalized, involving the superficial and deep nodes of the entire body (Table III). Of 280 cases, the state of the lymph nodes was mentioned in 210 (Table II). In 140, or 66.7 per cent, there was evidence of lymph node enlargement. These included 108 females and 32 males. We are inclined to view this figure as a conservative estimate of the degree of lymphadenopathy since in 55 of the remaining 140 cases (in which the nodes were not mentioned, or were said to be normal) necropsy was not performed. Tabulation of the enlarged nodes (Table III) shows the cervical chain to be involved most frequently, and the mesenteric, axillary, inguinal and retroperitoneal groups somewhat less so. In 12 per cent lymphadenopathy was generalized. The predominant involvement of the cervical chain of nodes may be more apparent than real; in some patients the cervical enlargement was noted clinically, and many of these patients were still living at the time of reporting,

or died and did not come to necropsy. Nor do we believe that the enlargement of the cervical nodes is related to the facial eruption. This premise would fail to explain the enlarged nodes elsewhere in the body, or the cervical lymphadenopathy in patients without skin lesions (case 2).

Morphologic Description

The enlarged nodes in disseminated lupus erythematosus are usually discrete, and vary in size from 1 cm. in the longest diameter to "large as a fist or even larger." They are soft and succulent, and enclosed

TABLE III
Distribution of Lymph Node Enlargement in 140 Cases of Disseminated
Lupus Erythematosus*

Group	Female	Male	Total
Cervical.....	47	13	60
Mesenteric.....	23	6	29
Axillary.....	18	7	25
Inguinal.....	16	8	24
Tracheobronchial.....	13	7	20
Generalized.....	14	3	17
Retroperitoneal.....	13	3	16
Supraclavicular and infraclavicular.....	7	3	10
Submaxillary.....	8	1	9
Pelvic.....	6	1	7
Epitrochlear.....	4	3	7
Para-aortic.....	5	1	6
Mediastinal.....	1	1	2
Submental.....	1	1	2
Site not stated.....	12	5	17

* The totals exceed the number of cases because in most instances more than one group of lymph nodes in the same patient was enlarged.

in a moderately tense capsule. The external surface has a pale gray, shiny appearance. The shape is oval, as a rule. Areas of necrosis of a yellowish or grayish white color may be recognized grossly, and sometimes there are "discrete foci of hemorrhage." Keil (1940) spoke of the "breakdown" of enlarged cervical lymph nodes "with the extrusion of necrotic, often infected, tissue" and likened their appearance to the so-called "scrofulous glands" of tuberculosis. On section, the cut surface of the nodes bulges from the tense capsule which encloses it. The pulp is edematous and almost mushy in consistency. The follicles and trabeculae are obscured in a gray, homogeneous background.

Microscopically, the marked distortion of the lymphoid architecture by edema and engorgement is the most prominent feature (Figs. 1 and 2). Neither primary nor secondary follicles remain. The lymph sinusoids are swollen and distended with lymphocytes, plasma cells.

histiocytes and reticulum cells (Fig. 3). The endothelial cells are also swollen and hyperplastic. Almost the entire vascular bed is engorged, and the capillaries bulge with red cells. The pulp is crowded with lymphocytes, and plasma, mononuclear and reticulum cells. Polymorphonuclear neutrophils are not numerous, and eosinophils are exceedingly rare. The mononuclear cells are swollen and have round, hyperchromatic, granular nuclei. Some contain cytoplasmic deposits of nuclear debris. A few reticulum cells may be undergoing mitotic division. Among the numerous plasma cells many have two or even three nuclei (Fig. 4). A constant finding is a large neutrophilic to eosinophilic cell, scattered sporadically throughout the pulp. It is three or four times as large as a lymphocyte, and is not unlike a megakaryocyte. The nucleus is large and multilobulated, and two or more nuclei may be present. The cytoplasm is homogeneous and free of vacuoles (Fig. 5). This cell is not to be confused with the Sternberg cell of Hodgkin's disease, or with the infectious mononucleosis cell described by Gall and Stout (1940) in the lymph nodes of that disease. The capsule, trabeculae and interstices of the node are not thickened, but isolated foci of fibroblastic proliferation may be noted. Some of the smaller arteries and arterioles are surrounded by a cuff of fibrous tissue, an alteration frequently observed around the central arterioles of the spleen (Fig. 6). These vessels may also show the distinctive changes of the disease usually seen in the viscera, particularly the kidneys.

In addition to hematoxylin and eosin, special stains were used on representative sections. No increase in reticulum fibers was found with the aid of Wilder's silver impregnation method, and Weigert's differential stain failed to disclose the presence of fibrin. Neither Masson's trichrome stain for connective tissue nor Mallory's phloxine and methylene blue stain revealed any features not noted in the hematoxylin and eosin preparation.

RELATED DATA

Spiethoff, in 1912, was the first to investigate the peripheral blood of patients afflicted with disseminated lupus erythematosus. He reported 2 cases, 27 and 20 years of age, respectively. In 1, the white cell count ranged from 3,400 to 4,600 cells per cmm., never rising above 5,300. In the other, the initial count was 6,100 white blood cells per cmm., rising to 6,950 soon after. It then fell to 5,100, rose to 5,600 and reached 9,450 just before death. In 1915, Spiethoff commented briefly on the absence of leukocytosis and the tendency to leukopenia in this disease. Subsequently this feature was noted by

others, more and more frequently, and undoubtedly is part of the syndrome of disseminated lupus erythematosus. Of 142 cases in which white blood counts were performed, 90 had leukopenia (less than 5,500 white blood cells per cmm.) at some time during the course of the illness. In many there was a distinct tendency towards leukocytosis during the terminal phase of the disease, contradicting Madden's statement that "the leucocyte count was lower just before death than at any other time." The white blood count usually hovers around 4,000 cells per cmm., occasionally falling below 3,000, or rising above 5,000. In Madden's sixth case, that of a girl of 20 years, the initial count of 4,000 white blood cells per cmm. rapidly fell to 1,200 and finally descended to 350. Although counts as low as this are not common, it tends to emphasize the degree of leukopenia which might be expected. The differential count usually has a normal distribution, and eosinophilia is not a feature. Friedberg and Gross (1936), and others, commented upon the association of thrombopenia with some cases of disseminated lupus erythematosus. These usually displayed moderately severe leukopenia, but the latter finding was by no means limited to the cases with thrombopenia.

Study of the bone marrow in disseminated lupus erythematosus has, perhaps, not received sufficient attention. The few comments on the state of the marrow, culled from the literature, are conflicting and inadequate. Certainly thrombopenia is not constantly associated with aplasia of the marrow. In Friedberg's second case (E. K.), which displayed marked thrombopenia (80,000), "the bone marrow from a vertebra showed activity, with many myeloid cells of all types." Templeton (1934) remarked that aplasia was noted in the marrow of his first case, in which the platelet count fell to 70,000. Baehr, Klemperer and Schifrin (1935) thought "perhaps due to toxic damage to bone marrow, or to vascular lesions in the marrow, the blood picture usually reveals evidences of a depression in bone marrow function, leukopenia, and a moderate anemia." In 1941, Klemperer, Pollack and Baehr reported 20 selected cases of disseminated lupus erythematosus and commented to the effect that "the bone marrow showed no changes of significance." Denzer and Blumenthal (1937) found the "bone marrow very cellular and the ratio of bone marrow cells normal" in their case. Ginzler and Fox (1940) said the red marrow in their case "showed definite hypoplasia of the blood-forming elements, though all were present in normal proportions. The marrow revealed no vascular alterations or areas of necrosis." A sternal biopsy in case no. 24201 of the Cabot series (1938) showed hyperplasia of the marrow. Banks (1941) believed that "there is often depression

of the bone marrow" in cases of disseminated lupus erythematosus, although in his case the marrow was not mentioned. The marrow (sternum) was examined in only 1 of our 3 cases (case 2) and failed to show any dyscrasia to account for the almost constant leukopenia.

Splenomegaly is not a feature of disseminated lupus erythematosus and "is best remembered in a negative way." In the cases reviewed in this paper, the spleen was mentioned specifically in 220, and in only 81 (36.8 per cent) of these was it enlarged. In none was the enlargement massive. Only 3 of the 20 cases of Klemperer, Pollack and Baehr had spleens which weighed over 300 gm. The average weight was 260 gm. In our 3 cases the spleens weighed 220, 240 and 218 gm. Except for frequent evidence of perisplenitis and focal infarction, there were no characteristic changes reported in the literature. A few authors noted miliary tubercles as part of a generalized state of miliary tuberculosis. Microscopically, two features were mentioned frequently, although not always in combination. These were areas of focal necrosis, similar to those described in the lymph nodes, and "a peculiar periarterial fibrosis limited to the central and penicilliary arteries." Klemperer, Pollack and Baehr (1941) gave prominence to the latter feature, having noted it in 19 of their 20 cases. "In cross sections of these vessels (central and penicilliary arteries) the fibrosis assumes a pattern of concentric rings of stout collagen fibers, with few intercalated fibroblasts." The necrotic areas are formed of poorly circumscribed zones of cellular débris. The nuclei of the cells which remain are pyknotic and karyorrhectic. At the periphery of such zones, the necrotic elements gradually blend with the surrounding nonnecrotic pulp, without forming any sharp line of demarcation.

REPORTS OF CASES

We wish to record 3 cases of disseminated lupus erythematosus, all of which had more or less generalized lymphadenopathy without evidence of tuberculosis. One case (L. P.) is of especial interest because of the absence of skin lesions (*lupus sine lupus*). It was the marked lymph node enlargement in this particular case which focused our attention upon the rôle of the lymph nodes in the disseminated form of lupus erythematosus.

Case 1

H. P. (N.B.G.H. 93782*), a white, native-born female, 24 years of age, enjoyed good health until 1937, when she developed a "crusting lesion" of the skin of the face. At that time she was suffering from "an infected tooth." A dermatologist con-

* We are indebted to Dr. John C. White of the New Britain General Hospital for permission to report this case.

sidered the skin condition to be "acute lupus erythematosus." She had had measles, mumps and whooping cough during childhood. No history of close contact with tuberculosis, or of rheumatic fever was elicited. The family history was noncontributory. Intramuscular injections of 3 per cent bismuth sodium tartrate were administered weekly until March, 1938, when the skin lesion disappeared. Three months later, a similar skin eruption appeared beneath the left eyelid and slowly spread to adjacent areas. Eighteen additional injections of the bismuth preparation were given without beneficial effect. In June, 1939, complete physical examination revealed a well developed and well nourished female with soft white skin. There was an erythematous crusting lesion over the bridge of the nose extending to both cheeks. The eyes and eyegrounds were normal. No abnormalities were noted in the heart or lungs and this was confirmed by x-ray examination. Blood pressure was 110/78 and pulse 85 per minute. The liver and spleen were not palpable. Rectal examination was negative. The tendon reflexes were equal and "very active." A small, nontender lymph node was felt in the right axilla. Roentgenograms of the teeth failed to show evidence of infection.

Laboratory tests at this time showed the hemoglobin to be 70 per cent (Newcomber), a "normal" urine and a negative flocculation test (Hinton). A tuberculin patch test was "faintly positive." In the spring of 1939, gold sodium thiosulfate and vitamins B and D were given; the patient was instructed to avoid exposure to sunlight. In addition, increasing doses of chaulmoogra oil were given intramuscularly, beginning with 1 cc., but were discontinued after the fourth injection because of a "painful, swollen, hot, indurated area which formed in the right buttock," associated with edema of the right lower extremity. Ten days after the last injection of chaulmoogra oil the patient developed pitting edema of both lower extremities, tenderness of the skin of the feet and ankles and a generalized maculopapular, pruritic eruption. Sulfanilamide, 20 grains, was given daily, for 6 days, without improvement. At this time enlarged, tender lymph nodes appeared in the epitrochlear, axillary and inguinal regions. The patient was admitted to the New Britain General Hospital on October 30, 1939, because of increasing weakness and malaise, associated with dizziness, swelling and tenderness of the right wrist and severe pain in the extremities. Her throat was "sore," and she had "bleeding gums." The temperature was 99° F.; pulse, 86 per minute; respiration, 20 per minute. There was a florid, crusted erythema of the forehead, nose and cheeks and a crusting, weeping eruption of the hands. The generalized rash had disappeared. The mucous membrane of the mouth was pale and the gums bled easily. Diffuse, sonorous râles were heard throughout both lung fields, without impairment of resonance or breath sounds. The heart was "normal." The spleen was not palpated, nor were there any swollen or tender lymph nodes at this time. The peripheral edema had disappeared, but marked tenderness was noted over the left heel, right wrist and right shoulder.

Laboratory Data. Hemoglobin, 66 per cent (10.0 gm.); red blood cells, 3 million per cmm.; white blood cells, 9,800 per cmm., with 65 per cent polymorphonuclear neutrophils, 31 per cent lymphocytes, 2 per cent eosinophils, 2 per cent monocytes. Sedimentation rate (Westergren), 120 mm. in 1 hour. Urine: specific gravity, 1.018; albumin, trace; red blood cells, few; occasional granular cast per low-power field. Transfusions of whole blood were given, and 10 days after admission to the hospital the hemoglobin was 69 per cent (10.5 gm.); red blood cells numbered 3 million per cmm.; white blood cells, 6,900 per cmm., with 63 per cent polymorphonuclear neutrophils, 34 per cent lymphocytes and 3 per cent eosinophils. Two days later the hemoglobin was 72 per cent (11.0 gm.); red blood cells, 4 million per cmm.; white blood cells, 6,500 per cmm., with 79 per cent polymorphonuclear neutrophils, 20 per cent lymphocytes and 1 per cent eosinophils. Two blood cultures were sterile. The blood nonprotein nitrogen was 123 mg. per cent and the creatinine, 2.5 mg. per cent.

Course. The temperature fluctuated between 99° and 103° F., rising to 105° on one occasion. Supportive treatment in the form of vitamin concentrates, diet and small repeated blood transfusions was given. Roentgenograms of the chest, 12 days following admission, revealed "bronchopneumonia" at the base of the left lung. Appetite diminished and somnolence increased, accompanied by involuntary twitchings. The patient died on the 17th hospital day.

Necropsy

The necropsy was performed 2 hours after death. Only the pertinent findings are recorded.

External Examination. Over the skin of the face there was a dry, scaly, blanched area with indefinite borders. It covered the bridge of the nose and both cheeks in butterfly fashion. Similar patches were found in the temporal regions, bilaterally, stopping abruptly at the hairline. There was no fissuring or loss of surface epithelium. A few similar patches of small size were found in the skin of the forehead, chest, and over the extensor surfaces of the arms, forearms and fingers. The skin of the entire back was peppered with slightly raised, punctate, maculopapules, most numerous over the bony thorax. The superficial lymph nodes were not palpable.

Internal Examination. The heart weighed 310 gm. There was no evidence of hypertrophy, thrombi, or valvular defects. The right and left lungs weighed 630 and 510 gm., respectively. The visceral pleura of the right lung was covered by strands of gray fibrin, especially over an accessory fourth lobe, situated between the right upper and middle lobes in the axillary line. It was solid and noncrepitant. The cut surface was engorged, gray-red and granular. Palpation disclosed numerous small, nodular areas, deep within the lung substance, which on section were slightly raised, bluish red, granular zones. Yellow pus exuded from the smaller bronchi and bronchioles in the centers of the granular areas mentioned above. These granular lesions were most numerous throughout the lower lobes of both lungs. There was no scarring or evidence of tuberculosis. The liver was swollen and weighed 1820 gm. It measured 30 by 21 by 7 cm. The external surface was mottled yellow-red; the cut surface was marked by scattered, small gray areas of necrosis, situated in a yellowish red background. The spleen was large and soft. It weighed 220 gm. and measured 14.5 by 8 by 3 cm. The capsule was smooth and tense; the cut surface bulged away from the capsule and revealed numerous, well defined, large gray malpighian corpuscles. At one pole there was a well circumscribed, necrotic gray zone, 1 cm. in diameter, as well as other smaller, similar zones. The pulp was deep red and soft. The right kidney weighed 220 gm. and measured 13 by 6.3 by 3 cm. The left

kidney weighed 230 gm. and measured 12.5 by 6.5 by 4 cm. A soft, wedge-shaped, gray-yellow zone was found in the cortex of the left kidney. The *lymph nodes* surrounding the aorta and those situated in the mesentery of the small intestine were all discretely enlarged, tense and succulent. The external surface was reddish pink, smooth and moist. On section the cut surface was homogeneously gray and moist. The follicle markings were obliterated, and there was no evidence of necrosis.

Microscopic Examination. No vascular changes or evidence of infiltration were noted in the *heart*. The bronchioles of the *lungs* were distended with an exudate consisting principally of polymorphonuclear cells, mixed with desquamated bronchiolar epithelium. The exudate extended from the bronchioles to involve the surrounding alveoli, and was also noted on the pleural surface. The malpighian corpuscles of the *spleen* showed extensive changes. The central arterioles were reduplicated and their walls were irregularly thickened. The outstanding finding, however, was thick collars of homogeneous, pink, acellular, periarterial connective tissue. The central arterioles were situated within these dense fibrous zones which occupied the major portion of the malpighian bodies. Some arterioles had deep red mural deposits of fibrinoid material, but the lumina were usually widely patent. A single large zone of necrosis, involving both the red and white pulp, was noted; at its periphery there was a wide band of hemorrhage and congestion. A small *accessory spleen* showed similar changes. There was no obvious scarring of the *renal cortex*, but the glomerular changes were very striking. Many endothelial cells lining Bowman's capsule were swollen, and there was rare capsular crescent formation. Bowman's space was filled with pink granular material in some places. Most of the glomerular tufts were free, although occasionally definite adhesions were present between them and the capsule. The prominent changes, however, were in the glomerular tufts, which were large and swollen. The swelling was not due to cellular proliferation, but rather to the deposition of deep pink-staining, collagenous tissue. Under subdued light, the connective tissue thickening of the basement membrane conformed to the typical "wire loop" design described in disseminated lupus erythematosus. Only rarely was a bright red focus of fibrinoid material seen within the glomerular tuft. The tubular epithelium was universally swollen and granular, and the lumina were filled with pink granular debris. No alteration of the afferent arterioles or medium-sized arteries was noted. A section of *skin* from a scaly lesion on the chest wall had a thin epidermis surmounted by a thin layer of keratin. Beneath the basal layer there

were scattered large mononuclear cells containing brown pigment, resembling hemosiderin. In addition, a small focus of lymphocytes and plasma cells was noted in the dermis. There was no sign of acute inflammation. All the *lymph nodes* examined were similar, but the structure of one node in the peripancreatic group was so completely disorganized that it was not possible to distinguish lymph follicles. This was due to the widespread edema which separated adjacent cells, associated with marked engorgement. The outstanding feature, however, was the diffuse increase in the number of reticulum cells. Areas of necrosis were not seen.

Anatomic Diagnosis. Scaly erythematous lesions of skin of face, anterior chest wall and arms; acute necrotizing arteriolitis of spleen and kidneys; hyperplasia of abdominal lymph nodes; focal pneumonia. (The anatomic findings were consistent with the clinical diagnosis of disseminated lupus erythematosus.)

Case 2

L. P. (N.B.G.H. 104075*), a white, Swedish female, 35 years old, was admitted to the New Britain General Hospital on April 22, 1941, complaining of "swelling of the glands of the neck." She stated that for 1½ years prior to admission she had had painful, symmetrical swelling of the middle joints of the fingers of both hands. During the past year she suffered from "arthritis of the neck, shoulders, spine and hips." Three months before admission she received "a course of gold injections" for the arthritic symptoms, without relief. She had the usual "childhood diseases." In 1935, an operation was performed for some gynecologic disorder, the nature of which was not known. The appendix was removed at that time. In 1937, she was treated for "an ulcer of the rectum." Her father died of "carcinoma of the pancreas"; her mother and seven siblings were living and well. Her husband and two children were also living and well.

Upon admission to the hospital the skin of the entire body was "clear." There was bilateral clubbing of the fingers and slight hypertrophy of the middle joints. Symmetrical nodular swelling of the preauricular, postauricular and superficial and deep cervical lymph nodes was noted. Roentgenograms of the chest showed "enlargement of the left auricle of the heart, and pulmonary congestion."

Laboratory Data. Hemoglobin, 57 per cent (8.7 gm.); red blood cells, 3.0 million per cmm.; white blood cells, 4,300 per cmm., with 68 per cent polymorphonuclear neutrophils, 27 per cent lymphocytes and 5 per cent monocytes. Sedimentation rate (Westergren method), 39 mm. in 1 hour. Urine: specific gravity, 1.006; albumin, +++; red blood cells, 175 to 200 per high-power field; 25 to 35 leukocytes per high-power field.

Course. Three days after admission a cervical lymph node was removed for diagnosis; it showed "lymphoid hyperplasia, nonspecific." Temperature ranged around 100° F., rising to 102.4° F. on one occasion. The pulse fluctuated between 70 and 90 per minute, respirations were 20 per minute and blood pressure was 162/100. The patient was discharged unimproved 4 days later.

Final Admission. The patient was readmitted to the New Britain General Hospital on May 20, 1941, complaining of a "choking sensation" beneath the lower

* We are indebted to Dr. Roger T. Scully of the New Britain General Hospital for permission to report this case.

sternum, associated with difficulty in breathing. These symptoms occurred at intervals of 3 to 4 days and would last for about 24 hours. There was intermittent nonproductive coughing, and on one occasion "rather profuse" hemoptysis. At this time the temperature was 99° F., pulse 90 per minute and respiration 20 per minute. Blood pressure was 120/90. A faint systolic, apical murmur was heard "occasionally." Multiple "petechiae" were found on the hard palate, and skin of the trunk. Bilateral costovertebral-angle tenderness was noted, and Murphy's sign was positive.

Laboratory Data. Hemoglobin, 40 per cent (6.1 gm.); red blood cells, 2.3 million per cmm.; white blood cells, 3,300 per cmm., with 63 per cent polymorphonuclear neutrophils, 32 per cent lymphocytes, 2 per cent eosinophils and 3 per cent monocytes. Sedimentation rate (Westergren), 70 mm. per hour. Urine: specific gravity, 1.004; albumin, ++; 125 to 150 red blood cells per high-power field; 20 to 25 leukocytes per high-power field; 6 to 7 granular casts per low-power field. The blood nonprotein nitrogen was 50 mg. per cent and the creatinine, 1.8 mg. per cent. Repeated blood cultures were sterile after 15 days.

The temperature ranged from 99° to 101° F. for 10 days, rising to 102° or 103° F. for a period of 1 week. It then fell to 99° F. for 5 days, only to rise precipitously to 103° F., reaching 104.6° F. on one occasion. From this time until death it continued at about 102° F. with minor fluctuations. The pulse rate more or less paralleled the temperature. The patient's condition became progressively worse. Numerous pyogenic cutaneous lesions appeared, particularly around the eyelids. At no time was any skin rash noted. She died on July 25, 1941, approximately 17 months after the onset of symptoms.

Necropsy

The necropsy was performed 9½ hours after death.

External Examination. The body was that of a well developed, but poorly nourished, white female of 35 years. The hair was reddish blonde; the skin was pale and clear; a few ecchymotic areas were found over the anterior chest wall and upper abdomen, but there were no petechiae. The posterior auricular and superficial cervical lymph nodes were moderately enlarged. The fingers were fusiform in shape, and clubbed.

Internal Examination. The right and left pleural cavities contained 200 and 100 cc. of clear, straw-colored fluid, respectively. The visceral pleura over the lower lobe of the *right lung* was partly covered by flakes of yellow fibrin. The right lung weighed 770 gm. The lower lobe was firm; the middle and upper lobes were crepitant and flabby. On section, the cut surface of the right lower lobe was reddish pink, and contained many poorly circumscribed, small gray-yellow areas. On pressure, thick yellow pus exuded from these areas. The *left lung* weighed 420 gm., and its external surface was smooth and glistening. Both the upper and lower lobes were crepitant. The *heart* weighed 270 gm. Except for slight thickening along the line of closure of the mitral valve, no valvular deformities were noted. The *peritoneal cavity* contained about 300 cc. of clear greenish fluid. The *appendix* was

absent. The *liver* weighed 1530 gm. and measured 23 by 19 by 7 cm. The *spleen* weighed 240 gm. and measured 14 by 9 by 6 cm. It was flabby in consistency; the capsule was bluish gray and wrinkled. On section, the cut surface was blue-red and studded with innumerable tiny gray flecks. The pulp could be scraped away easily. The *kidneys* were similar to each other. The right weighed 190 gm. and measured 13 by 6 by 3.5 cm.; the left weighed 220 gm. and measured 13 by 7 by 4 cm. The smooth, glistening capsules were stripped with ease, revealing a shiny, brick-red, lobulated cortical surface. Scattered over the cortical surfaces there were innumerable tiny hemorrhagic dots and streaks. On section, the cortex was 5 mm. in average thickness; the cut surface of both cortex and medulla was marked by hemorrhagic foci, similar to those on the external surface. The pelves and ureteropelvic junctions were not dilated. The mucosa of the slightly dilated *urinary bladder* was marked by several large, poorly circumscribed, hemorrhagic areas. The *pelvic organs* were all present and unaltered. The tracheobronchial, inguinal, pelvic and iliac groups of *lymph nodes* were enlarged, soft and succulent; the largest measured 2.5 cm. in longest diameter. The external surface was smooth, moist and reddish gray. On section, the cut surface had an edematous injected appearance. The lymph nodes in the region of the common bile duct and second portion of the duodenum, were also enlarged, as were those in the region of the stomach.

Microscopic Examination. The *mitral valve* was moderately thickened by loose cellular connective tissue in which there were small foci of lymphocytes. Near the base of the cusp the nuclei of the fibroblasts were arranged in palisade formation. Along the free border, opposite the area of palisading, there were a few "Anitschkow myocytes." Sections from the lower lobe of the *right lung* showed the alveoli to be completely filled with exudate composed of polymorphonuclear neutrophils, lymphocytes, mononuclear cells, fibrin and precipitated fluid. The lumina of the smaller bronchi and bronchioles were filled with a similar exudate. Isolated foci of Gram-positive cocci, arranged in chains, were found throughout the exudate. The malpighian corpuscles of the *spleen* appeared to be reduced in size and number. The walls of many of the central arterioles were thickened, without luminal narrowing, by homogeneous eosinophilic material. With Masson's trichrome method the inner portion of the vessel wall stained reddish purple and the outer portion green. Around some of the arterioles there was a wide cuff of fibrous connective tissue. The red pulp was engorged and diffusely infiltrated by polymorphonuclear neutrophils, lymphocytes, plasma cells and mononuclear cells. Several large cells

with single, round, or multilobulated hyperchromatic nuclei were also present. There were no areas of necrosis. Sections from both *kidneys* showed the glomeruli to be markedly altered by fusion of the tufts, both to each other and to Bowman's capsule. In a majority of the glomeruli, one or more, often two or three, tufts were converted into an eosinophilic granular necrotic mass in which all cellular detail was obscured. Portions of the conglomerate mass had a bluish hue. In some glomeruli, tufts at opposite poles showed these alterations and the intervening tufts were relatively unchanged. The basement membrane of many tufts was thickened so that the outlines were clearly visible, in sharp contrast to the surrounding, more or less indiscriminate mass. This accentuation of the basement membranes emphasized the "wire loop" picture described by Baehr, Klemperer and Schiffrin (1935). The capillaries of the uninvolved tufts were engorged, and surrounded by lymphocytes and polymorphonuclear neutrophils. Bowman's capsule, for the most part, was not thickened. However, in some places so much connective tissue thickening had occurred that the capsular space was completely obliterated and the glomeruli compressed. The afferent arterioles and juxtaglomerular bodies appeared to be unaltered. The interlobular and arcuate arteries were apparently spared. The intertubular stroma throughout both cortex and medulla was diffusely infiltrated by lymphocytes, plasma cells and a few polymorphonuclear neutrophils. The tubular cells were not altered although many tubules contained precipitated albuminous material. With Masson's trichrome method the eosinophilic glomerular masses stained red, suggesting necrosis. The thickened basement membrane of the glomerular tufts stained green, accentuating their "wire loop" appearance. The latter was also demonstrated by stains for elastic tissue and by van Gieson's mixture. In sections of the enlarged *lymph nodes* the architecture was distorted by marked engorgement of the blood vessels and edema of the lymph sinuses. Neither primary nor secondary lymph follicles were present. In addition to the lymphocytes usually seen in the pulp, there were innumerable plasma and large mononuclear cells. Some of the plasma cells had two nuclei. The nuclei of many of the large mononuclear cells were hyperchromatic, multilobed, and a few were undergoing mitosis; the cytoplasm was basophilic. There were also many reticulum cells. The wall of a small arteriole, in a section of inguinal lymph node, was thickened by eosinophilic hyaline material, like that described in the kidneys. A few arterioles in iliac nodes contained reddish blue granular plugs; their walls were thickened by connective tissue, with slight perivascular fibrosis. There was no apparent diminution

in the cellularity of the *sternal bone marrow* to account for the leukopenia noted clinically.

Anatomic Diagnoses. Primary. Acute necrotizing arteriolitis of kidneys and spleen; generalized enlargement of lymph nodes; fibrinous pleurisy and lobular pneumonia; acute splenic tumor; chronic cystitis; hydrothorax, bilateral; hydropericardium; ascites. *Subsidiary.* Fibrous thickening of mitral valve; scar of abdominal skin; absence of appendix. (The anatomic findings were consistent with the clinical diagnosis of disseminated lupus erythematosus sine lúpus.)

Case 3

L. M. (N.H.H. A76000*), white, American-born female, 16 years old, first developed "a pink wrinkled spot on the nose at the age of 7 years." When she was 14 years old the lesion, which had remained stationary until then, turned brown and became scaly. In February, 1937, at the age of 16 years, the posterior cervical and occipital lymph nodes became swollen. In May of that year, a barber noted that the patient had "eczema of the scalp and falling hair." One month later, red, swollen, itching patches developed on the cheeks and spread rapidly to involve the entire face except for the circumoral region. The eruption became scaly, soon extended to the shoulders and somewhat later to the upper thorax and arms. During this time swelling and pain in the "ankles, knees, hands and back" became manifest, and in August, 1937, easy fatigability, shortness of breath, nocturnal fever, anorexia, and red spots on the fingers and palms were noted.

The past history was noncontributory. Her mother had an area of pigmentation over the jaw, bilaterally, which had developed during pregnancy.

The patient was admitted to the New Haven Hospital for the first time on August 31, 1937. She had fever (103° F.), enlarged heart with tachycardia (124), gallop rhythm and incomplete atrioventricular block. There was generalized glandular enlargement. The spleen was not palpable. On September 7th the red blood cells numbered 2.87 million per cmm. with 62 per cent hemoglobin; white blood cells, 6,300 per cmm.; polymorphonuclears, 83 per cent; lymphocytes, 16 per cent; basophils, 1 per cent. After three blood transfusions the patient's serum agglutinated the cells of all prospective donors. Treatment consisted of liver, iron and, later, nicotinic acid. Two weeks after admission the skin lesion appeared to be receding and showed evidence of pigmentation. Three months later the eruption blossomed again, accompanied by hematuria, albuminuria, nausea, vomiting, ascites and pericardial effusion. The blood nonprotein nitrogen rose to 100 mg. per cent, but rapidly fell to 36 mg. per cent in 2 weeks' time. She was discharged from the hospital in April, 1938.

At home her condition seemed to improve, and she gained 10 lbs. in weight. During the latter part of May, 1938, however, she suddenly experienced severe stabbing precordial pain, precipitated by deep respiration. There was associated fever and weight loss, as well as recurrence of the skin lesion. She was readmitted to the hospital on January 14, 1938. At this time her heart measured 21 cm. in transverse diameter, as determined by roentgenograms, and the lower lobe of the left lung was "atelectatic." During her hospital stay the cardiac dimensions decreased slowly, and the skin lesion faded. She was discharged on August 12, 1938.

The patient was then ambulatory for approximately 6 months, during which time she was troubled occasionally by arthralgia of the knees and fingers. The precordial

* We wish to thank Dr. Francis G. Blake of the New Haven Hospital for permission to report this case.

pain and dyspnea gradually returned, as did the "rash of the face and hands." The third admission to the hospital was on March 11, 1939. The cardiac findings were similar to those of the previous admission, plus a systolic murmur in the mitral area. Following discharge on April 12, 1939, she suffered from "mumps and poison ivy."

She was readmitted to the hospital on December 29, 1939, because of swelling of the ankles of 1 month's duration. Hypertension (180/110) was noted for the first time; the cardiac findings were unchanged. The blood nonprotein nitrogen was 51 mg. per cent; serum protein, 4.26 per cent, with reversed albumin-globulin ratio; urinary albumin, ++++. On a salt-free, high protein diet the edema disappeared, and she was discharged on February 2, 1940. A few days later the edema reappeared and persisted. It was accompanied by dyspnea, orthopnea, anorexia, nausea, vomiting, epigastric discomfort, cough, restlessness, and thirst.

Final admission to the hospital was on February 23, 1940. Temperature was 100.2° F., pulse 100 per minute, respirations 24 per minute and blood pressure 144/112. On examination, the patient, who was now 19 years of age, was found to be chronically ill, with dry scaling, brown pigmentation of the face, distributed in butterfly pattern over the bridge of the nose. Elsewhere the skin was clear but dry. Signs of focal pneumonia were found. There was gallop rhythm, and a systolic murmur at the apex of the heart. The lower border of the liver extended 5 cm. below the right costal margin. The abdomen was distended and a fluid wave was elicited. There was pitting edema of the lower extremities and presacral region. The blood nonprotein nitrogen was 102 mg. per cent; serum protein, 5.8 per cent, with reversed albumin-globulin ratio and marked albuminuria. The sedimentation rate was not elevated. Digitalis and supportive therapy were given without relief. She became anuric on the fourth hospital day, and died 3 days later.

Necropsy

The necropsy was performed 1 hour and 40 minutes after death.

External Examination. The *skin* of the face was dry and scaly, with small, pale brown areas distributed in mottled fashion. The discoloration was absent around the alae nasae, over the chin, eyelids and base of the nose. The dryness and discoloration were also noted over the scalp, but stopped abruptly just beneath the mandible, did not extend over the neck nor over other parts of the body. The skin of the thorax and upper extremities was dry and loose; over the lower extremities it was tightly stretched, and there was moderate subcutaneous edema. There were no palpable lymph nodes in the cervical, supraclavicular, epitrochlear or inguinal regions; several small, firm, easily movable nodes were palpated in each axilla.

Internal Examination. The *pericardial cavity* contained 450 cc. of clear yellow fluid. There were numerous dense adhesions around the spleen and over the surface of the liver. The *heart*, together with the pericardium and bases of the great vessels, weighed 540 gm. The epicardium was thick and opaque. The pericardial space was completely obliterated by fibrous adhesions. The right atrium and ventricle were moderately dilated. The mitral valve was thickened and rolled along the free margin. The anterior cusp was quite short and bound

down to the ventricular endocardium by thin adhesions. The chordae tendineae of this cusp were thick and fused, as were the papillary muscles. The *right* and *left* lungs weighed 235 and 188 gm., respectively. Thick fibrous pleural adhesions were found. Each pleural cavity contained about 140 cc. of clear yellow fluid. Externally the lungs were pale pink, mottled by gray lines and flecks. The lungs were soft to palpation, except the left lower lobe, which was moderately crepitant. On the lateral aspect of the right upper lobe there was a brown-red, wedge-shaped area, 2 cm. in diameter. The *spleen* weighed 218 gm. and measured 12 by 7.5 by 4 cm. The external surface was dull purple, and the capsule was roughened by tags of fibrous tissue. The malpighian corpuscles stood out in the cut surface as small gray nodules, surrounded by soft pulp which could be scraped away easily. The *liver* weighed 1605 gm. and measured 25 by 19 by 8 cm. Each *kidney* weighed 180 gm. The right measured 11 by 6.5 by 4, and the left 10 by 7.5 by 4 cm. The capsules were stripped easily, revealing a mottled pink cortical surface, peppered by protruding, white, round areas, less than 1 mm. in diameter. Among these were small red dots, suggesting petechiae. The cut surface was similar to the external surface. The calyces, pelves and ureters were not dilated. There were several small, firm, easily movable nodes in the abdominal cavity and axillae; all showed evidence of pigmentation and hyperplasia. The *calvarium*, which transmitted light, contained considerable red marrow.

Microscopic Examination. A section of *heart* showed the small arteries of the myocardium to be markedly thickened so that the lumen was reduced to a narrow slit. However, the musculature was well preserved. Two small necrotic foci were present in which the muscle fibers were replaced by faint, pink-staining, granular material, surrounded by a few scattered lymphocytes. The smaller arterial branches in the *lung* sections revealed a striking degree of mural thickening, with marked reduction of the lumina. Sections from the right upper lobe showed foci of pneumonia. The malpighian corpuscles of the *spleen* were large. Their central arterioles had very thick walls of dense connective tissue, and many vessels were surrounded by increased fibrous tissue, in some cases involving most of the malpighian corpuscles. There were no necrotic foci. The small arteries of the *pancreas* also showed marked eccentric intimal thickening due to dense connective tissue. The *liver* was not remarkable. The small arteries in the *periadrenal* fat were thickened.

Marked changes were noted in the arteries and arterioles of the *kidneys*. The larger vessels showed advanced eccentric intimal thickening by loose connective tissue. The smaller arteries, however, were

altered by a necrotic process in varying stages of development. In some places the wall consisted almost entirely of bright, pink-staining, fibrinlike material, which was infiltrated by polymorphonuclear cells. Some vessels were rimmed by increased connective tissue and lymphocytes. Practically all glomeruli were surrounded by a thick ring of dense connective tissue, representing all stages of fibrotic change, including obliteration of the glomerulus; some were hyalinized. A few showed proliferation of Bowman's capsule, and many were unusually small. Isolated glomeruli were quite large, and their capillaries were engorged. The convoluted tubules varied strikingly, a cluster of them being widely dilated, and an adjacent group compressed by scar tissue. Many contained a small amount of pink granular material. The epithelium of the *urinary bladder* was not unusual. The muscle and subserosa contained small arteries which showed striking arteritis and periarteritis. One artery had a pinpoint lumen surrounded by a thick wall of granular necrotic material; the latter stained bright pink and had the appearance of fibrin. There were a few polymorphonuclear neutrophils in the wall, and perivascular clusters of lymphocytes. The wall of another vessel was composed of gray, necrotic, amorphous material.

The sinusoids of an abdominal *lymph node* were distended and relatively free of cells. Here and there eosinophils and large phagocytic cells were noted. The medullary cords and margins of the sinusoids were dotted by large numbers of mononuclear cells which were filled with brilliant brown pigment. The capillaries throughout the section were engorged. The *tongue* was the site of an acute inflammatory process; there were areas of necrosis and intense polymorphonuclear cellular infiltration in the superficial musculature. A section from the *rectus abdominus muscle* presented a small artery surrounded by densely packed lymphocytes. Some of the muscle fibers, seen only on cross section, stained bright pink; stippling, clearly seen in the less deeply stained fibers, was absent. The *skin* from the region of the left ear showed areas of brown pigmentation. The epidermis was thin, consisting of a basal layer, a narrow strip of stratum spinosum, and a single row of granular cells, surmounted by a rather thick layer of keratin. There was marked parakeratosis; almost all of the spinous layer consisted of thin, poorly demarcated, elongated cells with pink cytoplasm. In the dermis, close to the basal layer, there was a row of small mononuclear cells containing light brown granular pigment. Several small foci were seen in the subepithelial layers of the dermis in which the connective tissue stained deep pink and appeared hyalinized. There was moderate lymphocytic concen-

tration around some of the hair follicles and small arteries. Small hemorrhages were seen in the superficial portion of the mucosa of the colon. Many small arteries of the muscularis and subserosa had thickened walls and small lumina. One such vessel contained a large mass of connective tissue, probably an organized thrombus. The wall of this vessel was infiltrated by lymphocytes and plasma cells.

Anatomic Diagnosis. Primary. Acute necrotizing arteriolitis of kidneys; pigmentation, thickening and desquamation of skin of face and scalp; fibrous pericardial, pleural and peritoneal adhesions; cardiac hypertrophy; subcutaneous and cerebral edema; hydrothorax (bilateral); ascites; focal pneumonia (right upper lobe); pigmentation and hyperplasia of axillary and abdominal lymph nodes. *Subsidiary.* Fibrous thickening of mitral valve; ovarian cyst. (The anatomic findings were consistent with the clinical diagnosis of disseminated lupus erythematosus.)

SUMMARY AND CONCLUSION

This study was undertaken in an effort to determine the rôle of the lymph nodes in the syndrome of disseminated lupus erythematosus. A review was made of 277 reported cases, and 3 additional cases are reported in detail. The disease is most prevalent among white individuals between the ages of 11 and 40 years, with females predominating over males 3.5 to 1. Three Negroes, 1 Hawaiian of Japanese extraction, and 2 Puerto Ricans were included in the group. The lymph nodes were enlarged, either locally or generally, in 66.7 per cent of those cases in which they were specifically mentioned. The frequency of enlargement parallels the general incidence of the disease as to age and sex. The cervical group of nodes was enlarged most frequently, followed by the mesenteric, axillary, inguinal and retroperitoneal groups. The morphologic changes in the enlarged nodes are described in detail. The histologic picture is chiefly one of edema and engorgement, associated not infrequently with areas of necrosis. These features are considered suggestive of, but not specific for, the disease.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 10

- FIG. 1. Marked engorgement and loss of normal architecture in the enlarged lymph nodes. Hematoxylin and eosin stain. $\times 85$.
- FIG. 2. Edema of enlarged lymph nodes. Hematoxylin and eosin stain. $\times 250$.
- FIG. 3. Loss of normal architecture and cellular hyperplasia in enlarged lymph nodes. Hematoxylin and eosin stain. $\times 85$.

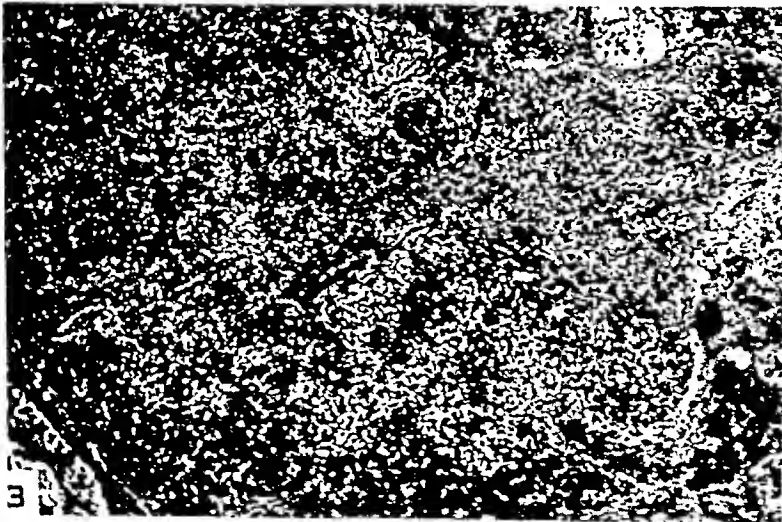
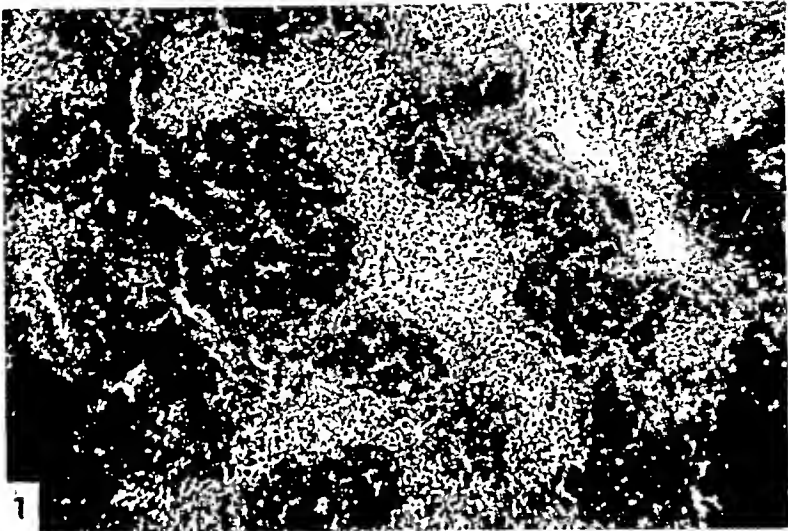
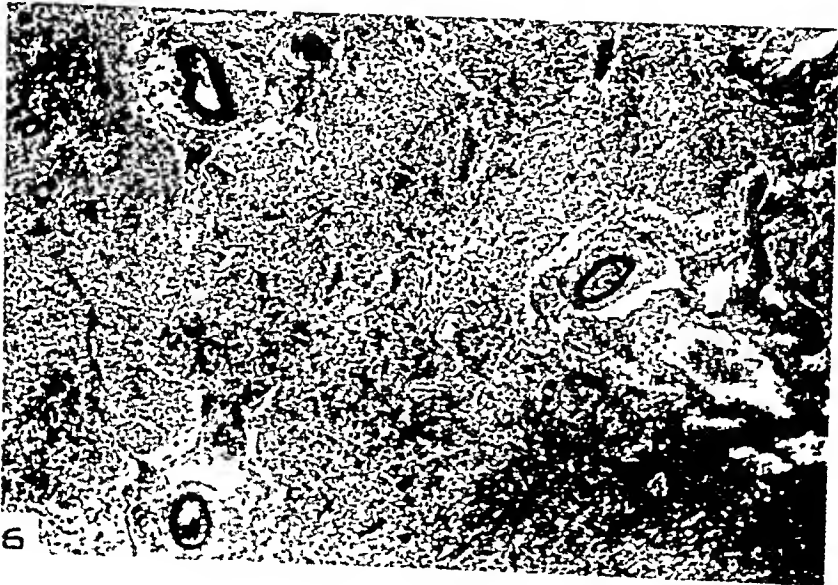
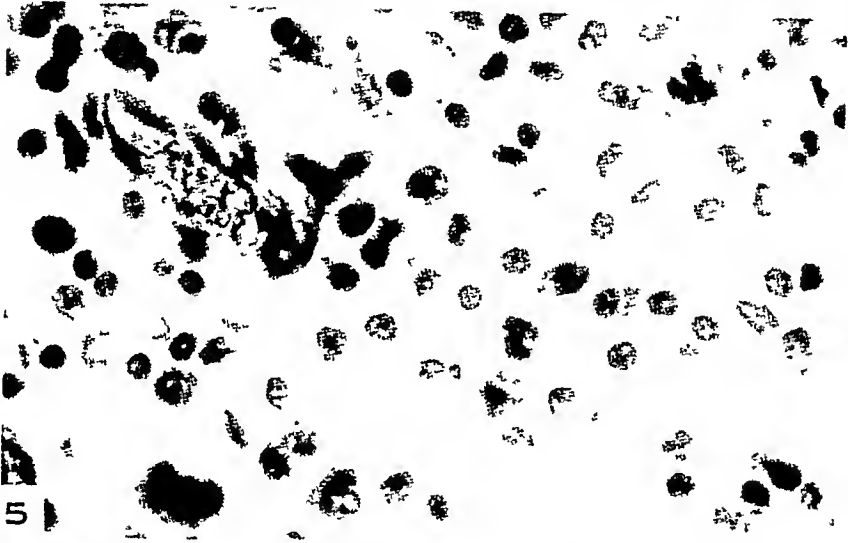
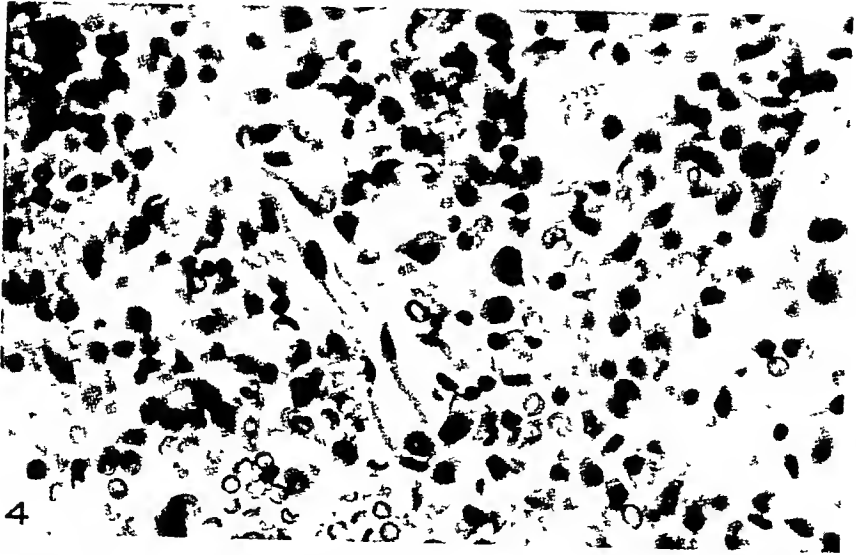


PLATE 11

FIG. 4. Numerous plasma cells, one of which has two nuclei. Hematoxylin and eosin stain. $\times 700$.

FIG. 5. Large cells with multilobed nuclei (megakaryocytes). Hematoxylin and eosin stain. $\times 775$.

FIG. 6. Small arteries with collars of connective tissue. Masson's trichrome stain. $\times 40$.



Fox and Rosahn

Lymph Nodes in Lupus Erythematosus



MEDIASTINAL SYMPATHOGONIOMA*

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A concise classification of tumors of the sympathetic nervous system based on their embryogenesis has been proposed by Bielschowsky.¹ The most immature formative elements of this order, the sympathogonia, show no neurofibrils. They have small, round, dark-staining nuclei enveloped in a delicate, homogeneous, and often indiscernible cytoplasm. These primitive structures are multipotential and give rise to both sympathetic ganglion and chromaffin cells. In the latter case they pass through the stage of pheochromoblast and pheochromocyte formation. In their development into sympathetic ganglion cells the first stage of ripening is the sympathoblast, a larger cell with a vesicular nucleus and rather abundant elongated protoplasmic processes. The end-state of maturation yields large multipolar forms with intracellular fibrils and Nissl substance—the sympathetic ganglion cells. Corresponding to each of these developmental steps are neoplasms composed of analogous histologic types. The cells in the most primitive tumor, the sympathogonioma, retain the lymphocytoid character of the parent stem cell though their arrangement may follow several patterns. Sympathoblastomas, derived from the ensuing subdivision, are made up of large, irregular, pear-shaped or oval cells with cytoplasmic streamers. The ganglioneuromas are characterized by large sympathetic ganglion cells incorporated in their fibrillar structure. In utilizing such a classification it must be borne in mind that these growths frequently are not composed purely of cells belonging to a single analogous plane of maturation but that considerable overlapping occurs. In evaluating the histologic picture the diagnosis must frequently be based on the predominating cell type.

Sympathetic tumors of all types are widely distributed throughout the body. In addition to their rather frequent appearance in the medulla of the adrenal glands, they have been reported as occurring in the abdominal, cervical, thoracic and pelvic sympathetics, the jejunum, celiac ganglion, mesentery, coccygeal gland, liver, uterus, cavity of the nose, skin or subcutaneum, and scapular region.² There appears to be adequate ground for the concept that peripherally mi-

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TABLE I
Intrathoracic Tumors Having Origin in the Sympathetic Nervous System

No.	Date	Author	Age	Sex	Location	Gross appearance	Metastasis or extension	Microscopic diagnosis
1	1870	Loretz ³	35 yrs.	F	Retropleural; lateral to left 2nd and 3rd thoracic vertebrae and adherent to 2nd and 3rd dorsal vertebral bodies	7.5 by 4.5 cm. in diameter; on section outer portion firm and soft; resembled uterine tissue	Extended into intervertebral foramina and the vertebral bodies	Ganglioneuroma
2	1897	Boerst ⁴			Retropleural; close to vertebral column	Almost size of two fists		Ganglioneuroma
3	1912	Friedrich ⁵	73 yrs.	F	Retropleural; between right 6th and 8th ribs and corresponding dorsal vertebral bodies	7 by 2.8 cm.; rather firm; cut section gray-yellow with fine fibrillar structure	None	Nonmyelinated ganglioneuroma
4	1923	Anderson and Shennan ⁶	12 wks.	F	Retropleural; region of right lung apex; attached to 2nd and 3rd ribs and sides of dorsal vertebrae	Ovoid, soft spongy mass 4.2 by 2.8 cm.; cut surface brownish pink, suggesting normal thyroid gland but darker	None	Neuroblastoma (small lymphoid cells and larger, sympathoblasts; rosette formation)
5	1924	Miller ⁷	39 yrs.	F	Retropleural; paravertebral at level of right 6th rib	Twice the size of a chestnut; weight 32 gm.; cut section ochre-yellow with occasional blood-red area; consistency spleenlike	None	Paraganglioma
6	1927	Cushing and Wolbach ⁸	2 yrs.	M	Paravertebral; springing from lamina or transverse process of 6th dorsal vertebra	"Plainly palpable and semi-fluctuant"	Extraspinal-dural extension through intervertebral foramen	Biopsy of original tumor, sympathicoblastoma; at operation 10 years later, original tumor had disappeared; remaining extradural extension, a ganglioneuroma

7	1927	Capaldi ⁹ (Case 1)	44 yrs.	F	First tumor lateral to left C7 and D1 vertebrae, attached to 2nd rib below Second tumor on corresponding right side attached to first rib Third tumor beneath left adrenal	Size of pigeon's egg, firm, gray-white Bean-sized; similar appearance Size of small apple	Extension to spinal cord dura and into brachial plexus Extension to spinal cord dura	Neuroblastoma simplex (immature sympathoblastoma) Neuroblastoma simplex (immature sympathoblastoma) Neuroblastoma simplex (immature sympathoblastoma)
8	1927	Capaldi ⁹ (Case 2)	2 yrs.	F	Retropleural with massive extension into left upper thorax, cervical and axillary regions	Knobby, firm, gray-white tissue mass	Penetration of intervertebral foramina with extradural extension from D8 to medulla oblongata	Well differentiated ganglioneuroma
9	1932	Millar ¹⁰	34 yrs.	M	Retropleural; adherent to left 6th, 7th, 8th thoracic vertebrae and penetrating left lung	On section, intensely black inner circular portion about 3 cm. in diameter surrounded by pinkish fleshy zone 6 cm. in diameter	Extension to lung, thoracic vertebral bodies and into spinal canal; metastases to ribs, skull, right adrenal, right pectoral muscle and right ear	Malignant melanotic ganglion cell tumor
10	1932	Scott and Palmer ²	22 mos.	M	Retropleural; posterior to lower lobe of left lung	Lobulated, encapsulated mass 13 by 13 by 7.5 cm., weighing 572 gm.; cut section finely granular	Extension to right pleural cavity and extradurally over spinal cord	Sympathicoblastoma (sympathogonia and sympathoblasts; rosette formation)

TABLE I continued

No.	Date	Author	Age	Sex	Location	Gross appearance	Metastasis or extension	Microscopic diagnosis
11	1936	Frost and Wolpaw ¹¹	38 yrs.	M	Retropleural at apex of right thorax with extension into upper lobe of lung	9 by 5.5 by 7 cm.; irregularly nodular and not encapsulated; cut section dense, gray with somewhat whorled appearance	Extension into esophagus, right lung, last cervical vertebra and first 3 ribs; metastasis to right adrenal, kidney, jejunum, axillary nodes and subcutaneous tissue	Sympathoblastoma
12	1937	Stern and News ¹²	7 yrs.	F	Posterior mediastinum with forward displacement of aorta and lobe of right lung	Not given	Extension to right lower lobe of lung and infiltration of the epidural space of spinal cord; bilateral pleural metastases	"Sympathogonioblastoma" (mixed sympathogonia and sympathoblasts)
13	1940	Philips ¹³	39 yrs.	M	"Apex of left pleural cavity not attached to lung"	Size of plum, easily shelled out; on section alternating firm white, and soft hemorrhagic purple areas	None	Pheochromocytoma

grating primitive nerve cells of the developing sympathetic nervous system may come to rest almost anywhere in the body and are capable of proliferating and forming tumors at a later date. Table I briefly summarizes some of the salient features of 13 well documented sympathetic tumors recorded as occurring within the thorax.

The tumor to be described is of singular interest because of its peculiar location in the anterior mediastinum, and its striking histologic composition. The occurrence in a patient of advanced age and the biologic behavior of a growth of such a primitive type are also very unusual features.

Report of Case

M. C., a colored female, 65 years of age, was brought by ambulance to the accident room of the Cincinnati General Hospital on November 20, 1940, in a semicomatose state. According to the patient's son, 4 days before admission she had complained of severe headache and dizziness, accompanied by progressive general weakness. Chills, fever, vomiting, or other associated symptoms had not been observed. The patient became drowsy and stuporous shortly before admission. It was learned that during the past 2 years there had been occasional attacks of shortness of breath and precordial pain. A year before, she had been confined to bed because of congestive heart failure and at the time exhibited considerable swelling of her legs and abdomen. After 2 months she had recovered sufficiently to be able to perform housework with only moderate dyspnea and had been in fair health until the onset of her present illness. No other relevant facts were obtained from the history. Physical examination on admission showed a semicomatose, elderly Negress, who responded incoherently and indifferently to questioning. Temperature, 101.5° F.; pulse, 114; respiration, 34; blood pressure, 185/108. Her neck was rigid in all directions. Kernig's and Babinski's signs were positive. Deep reflexes were hypoactive in the upper extremities. Knee jerks were absent in both lower extremities. Consolidation was present at the base of the left lung, posteriorly. A spinal puncture was performed and a small amount of milky fluid under increased pressure was removed. The white blood cell count of the spinal fluid was 17,600 with 98 per cent polymorphonuclear neutrophils. *Haemophilus influenzae* was found on smear and culture. Blood culture was also positive for *H. influenzae*. The blood Kahn was negative. Sulfapyridine therapy was instituted but proved to be ineffectual and the patient succumbed 2 days after admission.

Autopsy Findings. An abstract of the positive findings at autopsy, performed 18 hours after death, follows:

The patient was a moderately obese, colored female appearing the stated age of 65 years. Herpetic lesions were present on both lips. The upper jaw was edentulous and the remaining lower teeth carious. There was bilateral arcus senilis. A few fibrous adhesions bound the apex of the right lung and the diaphragmatic surfaces of both lower lobes to the parietal pleurae. The medial half of the right transverse pulmonary fissure was incompletely developed and the oblique fissure posteriorly on the same side was absent. Emphysematous bullae were present over the anterior margin of the right middle lobe. The left

lower lobe was increased in consistency and had a dark red, moist, slightly granular appearance on section. The remaining lobes were dry, inelastic and hypercrepitant except in the dependent portion where they were moist and poorly aerated. Projecting into the anterior superior mediastinum, overlying the superior vena cava and attached laterally by adhesions to the first portion of the aorta, was a round, firm mass, 5 cm. in diameter. This was covered by the pericardial sac whose upper portion was bound rather firmly to the anterior and lateral surfaces of the tumor by old fibrous adhesions. Easily broken, fine, white fibrous bands fastened the adherent pericardium to the mediastinal surface of the right lung. On stripping away the pericardium, the tumor was found to be well encapsulated and rather loosely attached by fibrous adhesions to the anterior surface of the superior vena cava and the upper portion of the markedly dilated right atrium, lying above and some distance anteriorly to the right pulmonary artery and eparterial bronchus (Fig. 1). No nerve fibers or ganglia were seen entering its capsule. The cut surface of the tumor was uniformly reddish brown; its consistency soft. Elsewhere both layers of the pericardial sac were lightly bound together by delicate fibrous strands. Both cardiac ventricles were slightly dilated. The musculature was uniformly pale reddish brown and soft, left ventricular wall thickened and the endocardial surfaces of all the chambers were smooth and glistening. Raised yellow intimal plaques were present throughout the aorta. In the region of the arch these were partially calcified. The abdominal organs were in normal anatomic relation to one another. Eight small black calculi were present in the fundus of the gallbladder. The gallbladder wall was thin and pliable. The adrenal glands were intact. A circumscribed, firm, myomatous tumor within the wall of the uterine fundus measured 3 cm. in diameter. Several soft red polyps were present in the uterine cavity. A round, multilocular serous cyst, 8 cm. in diameter, occupied the left ovary.

The brain weighed 1275 gm. A thick, greenish, fibrinopurulent exudate distended the subarachnoid space over the convex surfaces of both cerebral and cerebellar hemispheres and collected at the base of the brain in the region of the optic chiasm. A similar exudate involved the leptomeninges of the spinal cord.

Microscopic Findings. Numerous sections taken throughout the mediastinal tumor showed a strikingly uniform cell type and composition. The dense, enveloping, fibrous connective tissue capsule contained focal deposits of calcium salts. One section cut near the posterior surface showed a few nests of tumor cells penetrating the

capsule and infiltrating an adherent tag of adipose tissue. No other areas of capsule invasion were found. Stained with hematoxylin and eosin, the tumor cells in practically all sections appeared to consist entirely of nuclei. These were predominantly round or oval, rich in chromatin and ranged in size from one to two times the diameter of a red blood cell. Stained with Masson's trichrome stain, however, a delicate enveloping rim of vermilion-red cytoplasm could be demonstrated about most of the prominent purple-black nuclei. No cytoplasmic granules were seen in Zenker's fluid-fixed material. In some instances the cytoplasm assumed a bipolar arrangement, streaking out at each end of the nucleus in a wedge-shaped pattern for a distance equal to or surpassing the greatest diameter of the nucleus (Fig. 2). Others showed the base of the cytoplasmic wedge resting on only one pole of the nucleus, thence abruptly narrowing to an elongated fine thread which was attached to an adjacent cell border or a blood vessel. While the tumor possessed a rather rich capillary network, only rarely were such threadlike processes found approaching the vessel wall at right angles, the cells usually appearing parallel to the vessel axis (Fig. 3). The modification by Foot and Foot of Bielschowsky's silver stain showed the cell nuclei to be intensely argentophilic, but only very rarely and by repeated sectioning could a reticulum fiber be located. Intracellular or extracellular neurofibrils could not be demonstrated with Bodian's protargol stain. The majority of the cells were of the small, round, lymphocytoid type (Fig. 4), and there were but few attempts at rosette formation; where present, these lacked the circular arrangement of the cells about a central limiting membrane considered by Bailey and Cushing¹⁴ typical of true rosettes (Figs. 5 and 6). An occasional field showed whorl-like masses constructed of closely packed, short, oval cells (Fig. 7). Under higher magnification some of these nuclei were slightly indented on their lateral surfaces or diminished in thickness toward one pole exhibiting a carrot shape suggesting the formation of lemmoblasts. Further support for assuming a deviation in this direction of growth was brought out in a rare zone in which there was an interlacing bundlelike arrangement of elongated spindle cells such as is frequently encountered in neurilemmomas of peripheral nerves (Figs. 8 and 9). In sections stained with hematoxylin and van Gieson's mixture there were only a few isolated zones in which collagenous supporting fibers were found.

In addition to this neoplasm in the anterior mediastinum, examination of the remaining organs showed a chromophobe adenoma, measuring 7 by 6 mm. in the anterior lobe of the pituitary gland; a small mucosal adenomatous polyp of the fundus of the stomach; multiple

intramural leiomyomata of the fundus uteri; several adenomatous endometrial polypi, and a multilocular serous cyst of the right ovary.

Pathologic Diagnoses. The final pathologic diagnoses were: acute influenzal cerebrospinal meningitis; bronchopneumonia, lower lobe of the left lung; sympathogonioma of anterior mediastinum originating beneath the pericardium, probably from within the sympathetic rami of the deep cardiac plexus; chronic obliterative pericarditis; slight myocardial hypertrophy and dilatation; focal myocardial scarring; chronic pulmonary emphysema; pleural adhesions; chromophobe adenoma of the anterior lobe of the pituitary gland; adenomatous polyp of the gastric mucosa; multiple intramural leiomyomata of the uterus; endometrial polypi; multilocular serous cyst of the right ovary; cholelithiasis; chronic cholecystitis; aortic atherosclerosis with focal calcification; moderate generalized arteriosclerosis.

DISCUSSION

Among the intrathoracic sympathetic tumors listed in Table I, two examples are of the chromaffin cell type, the remaining falling under the heading of sympathetic tumors in the strict sense of the word. In almost all of the latter the primary site was behind the parietal pleura with growth anteriorly into the thoracic viscera. In many, direct continuity with the sympathetic chain was anatomically demonstrable. None of these tumors exhibited either a location or histologic structure comparable to that in the case presented. In this tumor, the uniform cell plan observed throughout the numerous sections leaves little doubt of its proper classification according to Bielschowsky's¹ outline. Absence of maturation into larger sympathoblastic cells further indicates its embryonal character, although the presence of occasional scattered small cells with unipolar and bipolar cytoplasmic processes may represent an intermediate step in this direction. A few areas which contain carrot-shaped forms suggest the production of lemmoblasts, and the occasional interlacing bundles of elongated spindle cells are in keeping with a later development along this line. Earlier investigations on the embryologic development of the sympathetic system in mammals corroborate such a formation of Schwann cells from the immature anlagen of sympathetic ganglia.¹⁵ The location of the tumor beneath the pericardium over the right atrium indicates a probable derivation from embryonal sympathetic elements contained in the deep cardiac plexus.

The complete encapsulation of the tumor with only one microscopic field displaying penetration of tumor cells into the surrounding tissue; together with the absence of distant metastases, seems to belie the

rapid growth which such immature cell types would indicate. Similar growths appearing elsewhere in the body are usually characterized by early and widespread metastases. Why such a protracted growth rate is present in this case is not clear, and certainly the anatomic location of the tumor could not influence its capacity to grow. The appearance of such a tumor in a patient in the sixth decade of life is also unusual, though poorly differentiated sympathetic neoplasms in other locations have been described as occurring in late adult life.^{16, 17} One can readily understand how more widely disseminated neoplasms of the mediastinum and lungs, designated as lymphosarcomas, thymomas or small-cell carcinomas, might be confused with embryonal tumors of the sympathetic nervous system in this location, once growth and dissemination had become active.

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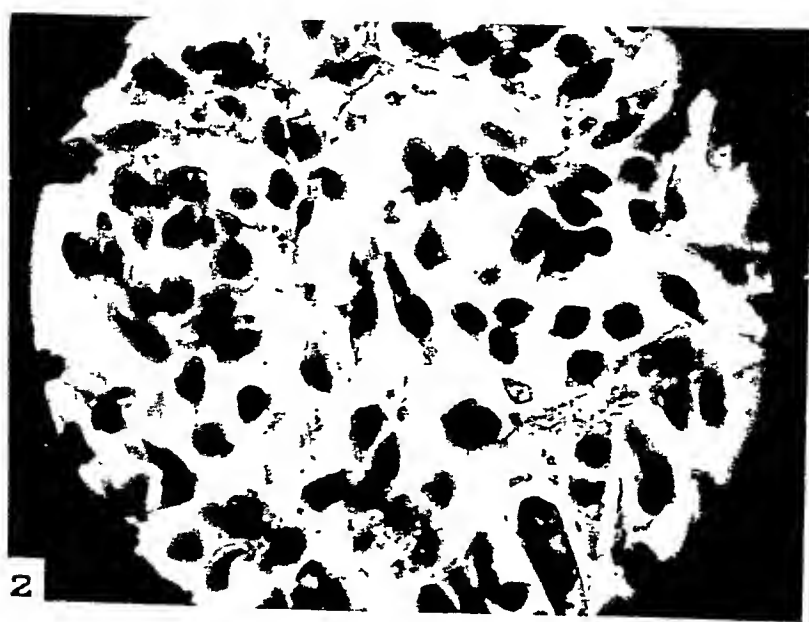
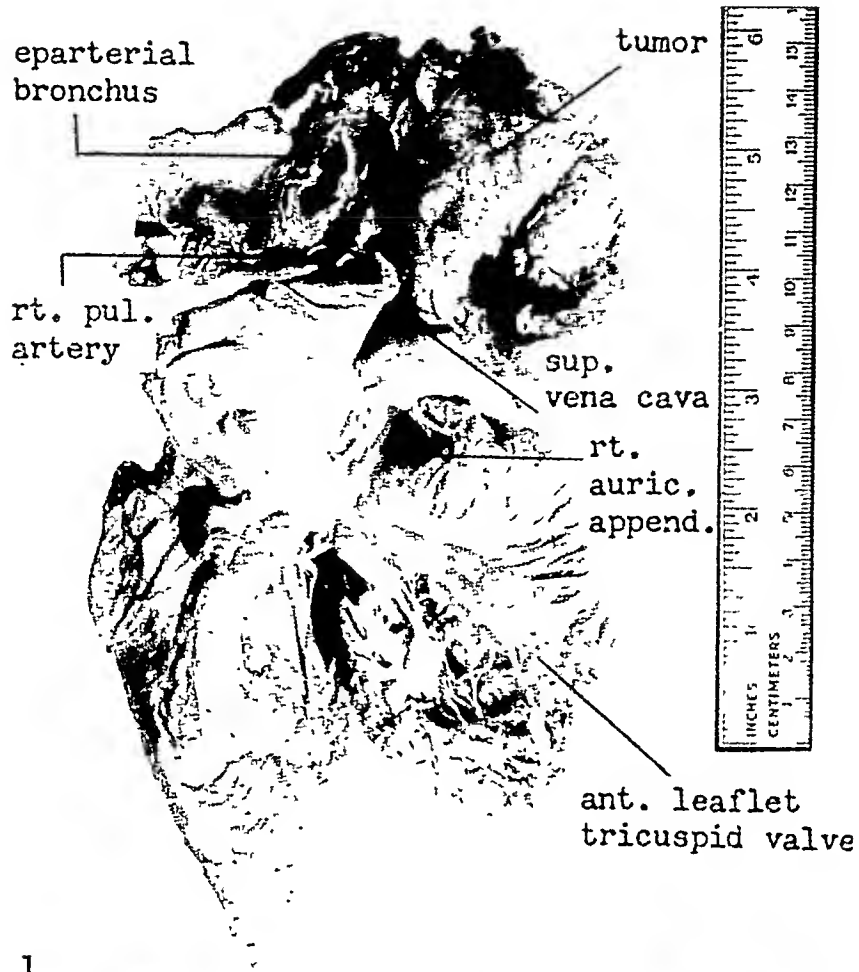
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DESCRIPTION OF PLATES

PLATE 12

FIG. 1. Relation of tumor to opened right atrium and associated structures.

FIG. 2. Bipolar arrangement of cytoplasm about oval nucleus. Masson's trichrome stain. $\times 1125$.



Sailer

Mediastinal Sympathogonioma

PLATE 13

FIG. 3. Filamentous cytoplasmic streamer attached to capillary wall. Masson's trichrome stain. $\times 2250$.

FIG. 4. Compact area of uniformly round cells. Hematoxylin and eosin stain. $\times 160$.

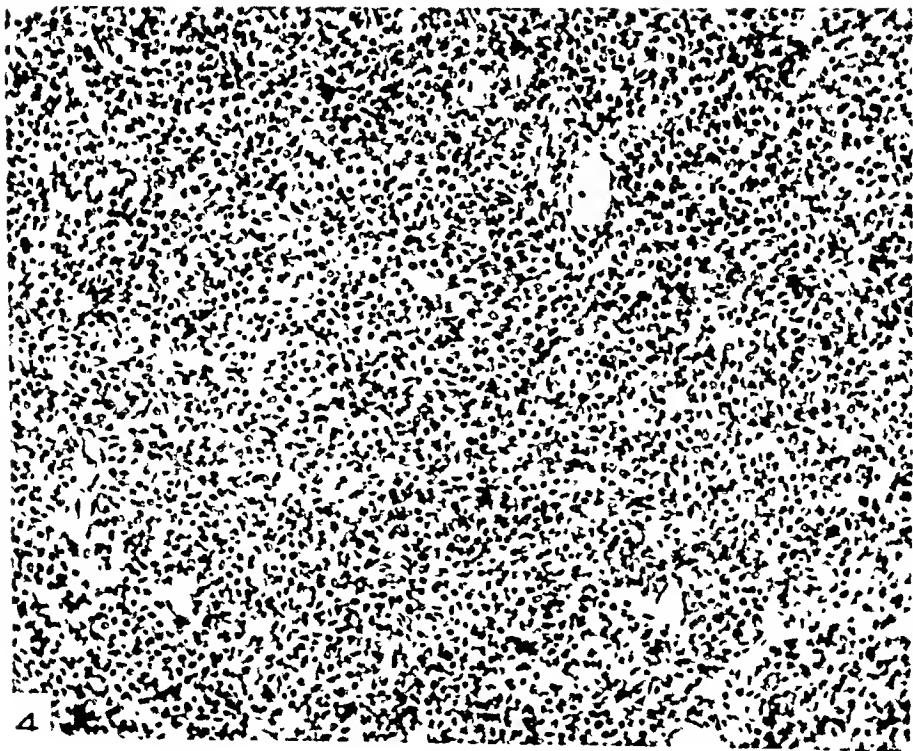
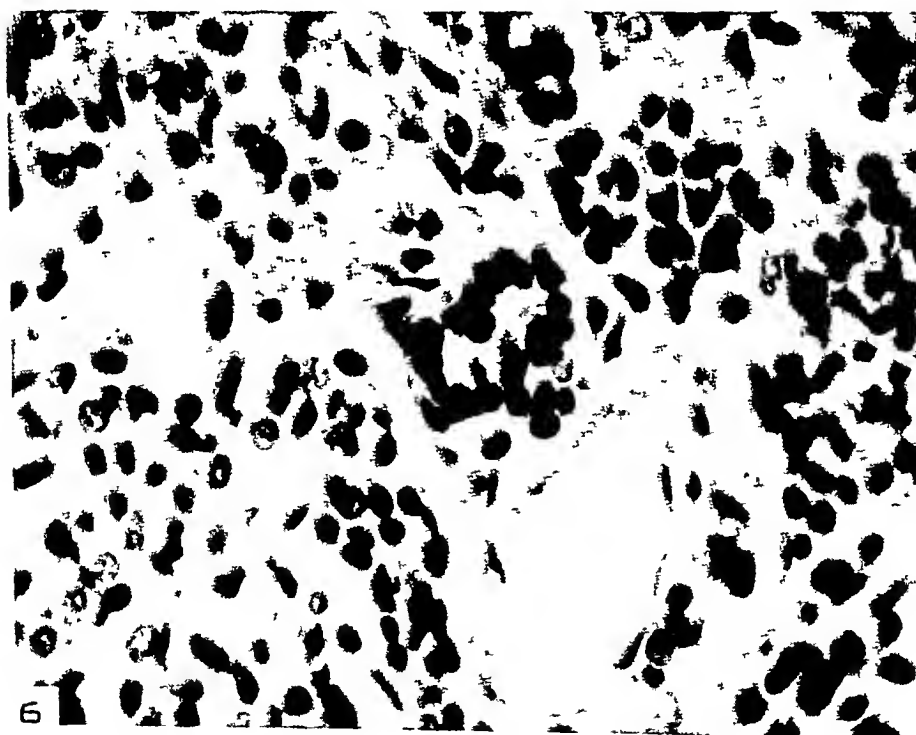
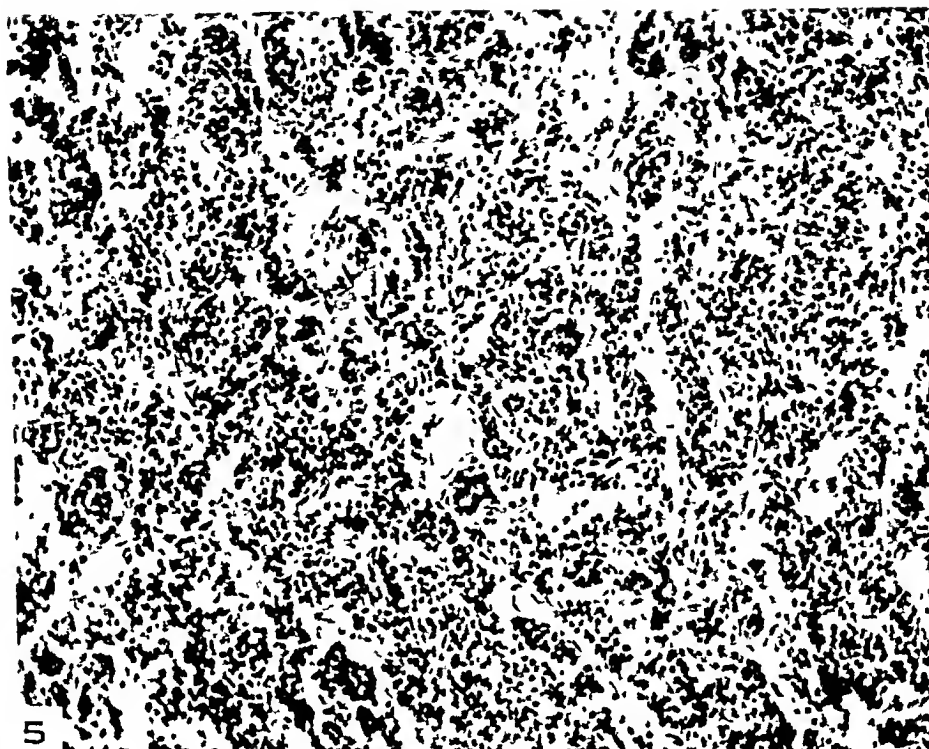


PLATE 14

FIG. 5. Areas showing pseudorosette formation. Hematoxylin and eosin stain.
× 160.

FIG. 6. Areas showing pseudorosette formation. Hematoxylin and eosin stain.
× 600.

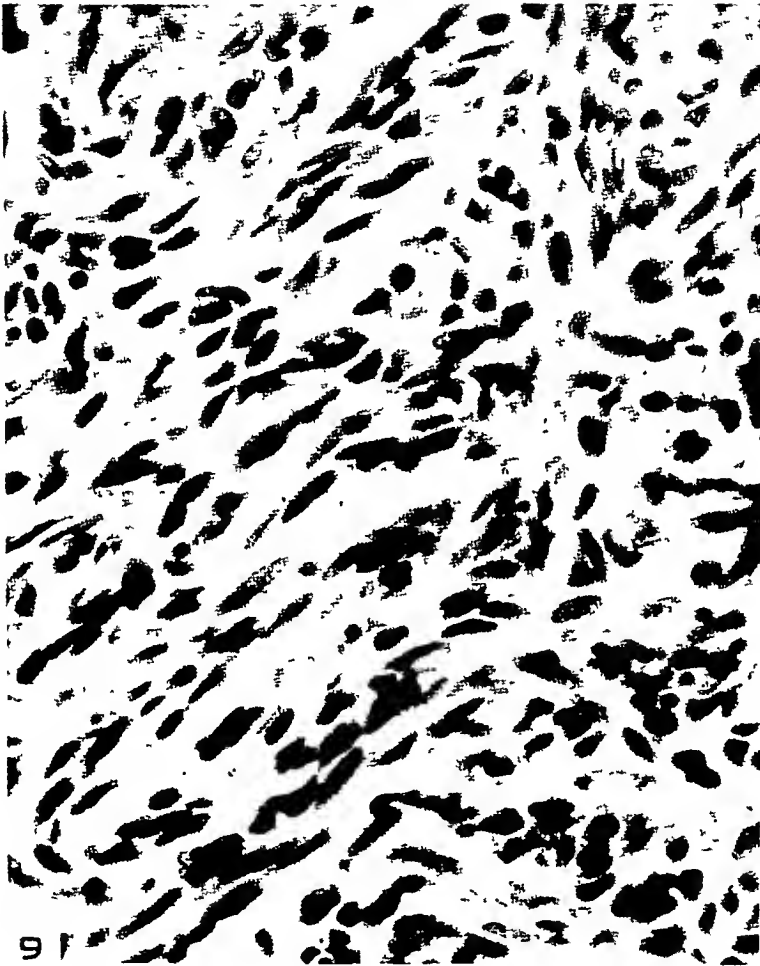


Sailer

Mediastinal Sympathogonioma

PLATE 16

FIG. 9. Interlacing bundles of elongated spindle cells. Hematoxylin and eosin stain.
× 600.



Sailer

Medialastinal Sympathogonioma



MEDIAL HYPERTROPHY OF THE RENAL ARTERIOLES IN PREGNANCY *

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In the recent European literature much attention has been given to the special mode of termination of renal afferent arterioles in man and many other mammals. Smith¹ in his Harvey Lecture has reviewed the historical development of our knowledge of this subject and the reader may get fuller details there. For purposes of this report, it may suffice to summarize this literature as follows: A number of observers (Clara,² Becher,³ Goormaghtigh,⁴⁻⁶ Benninghoff⁷) have stressed the occurrence of nonfibrillar, "clear" smooth muscle cells in the media of normal vessels, and some^{4, 6, 8, 9} have described granular chromophilic cells in the termination of the afferent arterioles in some species. Some regard them as related modifications, and both types as modified smooth muscle cells. Zimmermann¹⁰ described, as well, the peculiar expansion of the terminal portion of the afferent arteriole due to an increase in medial cells, which he designated as the "Polkissen" (polar pad). This may be eccentric or concentric. Goormaghtigh has designated these groups of cells as the "juxtaglomerular apparatus." He¹¹⁻¹³ has recently claimed that in renal disease in man and in experimental renal hypertension in dogs and rabbits the clear cells and granular cells multiply and undergo hypertrophy. To them he has assigned an endocrine rôle in the formation of the pressor substances probably responsible for renal hypertension. Dunihue and Candon¹⁴ have confirmed these claims in rabbits.

A nephrectomy performed for the relief of intermittent hematuria of at least 3 years' duration in a colored female, 29 years old, revealed unusual vascular lesions of the kidney. Because of the remarkable transformation of the media of the afferent arterioles and the enlargement of the "Polkissen" encountered in the kidney in the absence of hypertension and during pregnancy, this case seems worthy of publication. The subsequent development of eclampsia and mild hypertension lend special interest to the vascular changes to be described.

Report of Case

E. M., a colored female, 29 years of age, was first admitted to the Urological Service of Bellevue Hospital in April, 1938. At that time she complained of painless hematuria of 1 year's duration. There had been a slight loss of weight (8 lbs.). There were no other complaints. She reported that she had been treated at another

* Received for publication, April 13, 1942.

hospital for the same complaint 3 months before entering Bellevue Hospital. At unstated periods she had previously received injections for syphilis, and in 1932 she had had a spontaneous miscarriage.

Physical examination revealed no signs of organic disease. The patient did not appear to be ill. Some observers thought there was an indefinite mass in the left upper quadrant, possibly the spleen or kidney. The blood pressure on this admission was 116/68 mm. Hg. There was moderate anemia with 45 per cent hemoglobin, 3.4 million erythrocytes per cmm., 4,000 leukocytes per cmm., with 81 per cent polymorphonuclear leukocytes. Blood nonprotein nitrogen was 34 mg. per 100 cc. Cystoscopy revealed a normal bladder, but blood could be seen trickling from the left ureteral orifice. Intravenous pyelography revealed no abnormalities; there was no record of urine culture. A left-sided abscess found in Bartholin's gland was incised. Culture of the pus yielded a staphylococcus, Gram-positive bacilli and diphtheroids.

With these findings, some urologists believed that hematuria might have been the result of infection and inflammatory changes, and the patient was given urinary antiseptic drugs. Following the administration of iron by mouth, the erythrocytes rose to 5 million per cmm. and the hemoglobin to 70 per cent.

However, the hematuria continued. The patient was readmitted in August and again in October, 1938, and the same findings were reported. Cystoscopic and pyelographic investigations were repeated with the same results. Intravenous injection of methylene blue showed that the dye appeared in the left ureter a little earlier (5 minutes) than on the right side (8 minutes). No other renal function tests were done. Blood nonprotein nitrogen was within normal limits.

The fifth and sixth admissions occurred in November and December, 1939, and at this time the patient was pregnant. In the latter admission she was estimated to be in the third month of gestation. Urinary findings persisted as before. Cystoscopy was repeated and a tissue specimen of each ureteral orifice was taken and reported to show chronic inflammation. There was moderately severe anemia, and the patient was given two blood transfusions. Erythrocytes rose from 2.0 to 3.9 million per cmm. and the hemoglobin from 30 to 60 per cent.

Members of the Obstetrical Service believed that the pregnancy should not be interrupted. The urologists now considered nephrectomy advisable, for a neoplasm was believed to be the most probable cause of the hematuria. Consequently, on February 23, 1940, a left nephrectomy was performed and the patient made an uneventful recovery. Pregnancy was not disturbed. There was no further hematuria. The blood pressure before and for a week after operation ranged from 110 to 114 systolic and from 68 to 70 diastolic. The kidney will be described later in this report.

Postoperative and Antepartum Course. Special renal function tests were performed 2 weeks after operation and at intervals thereafter. They are referred to below. The blood pressure remained normal until May 13, 1940, when in the ninth month of gestation it rose to 140/96. The urine showed a trace of albumin which persisted for the next 2 weeks. At the patient's last visit to the Prenatal Clinic on May 28th the blood pressure was 130/78 and the urine was normal.

On the night of May 30, 1940, in the ninth month of gestation, she had two convulsive seizures at home and was taken to Harlem Hospital in a semistuporous condition. On physical examination slight edema of the ankles was noted and the blood pressure was found to be 140/90 mm. Hg. One hour after admission a spinal tap was done. Clear fluid, 35 cc. in amount, was removed under increased tension. There was another convulsion and the patient went into a deep coma. Chemical study of the blood showed creatinine, 1.4 mg. per cent; urea nitrogen, 14 mg. per cent; sugar, 120 mg. per cent, and uric acid, 10.5 mg. per cent. Urine obtained by catheter contained albumin, 4 plus; and microscopic examination showed many erythrocytes and occasional granular and hyaline casts.

Labor. Coma persisted and spontaneous labor began 16 hours after admission. The first stage lasted 14 hours, the second stage 25 minutes, and the patient spontaneously delivered a stillborn male child. During labor the blood pressure was 175/105 mm. Hg, the temperature was 104° F. and the pulse was 140; respiration numbered 26 per minute.

Puerperium. The patient came out of coma 16 hours after delivery and began to void large amounts of urine frequently.

Blood Pressure Readings During the First 10 Days Postpartum

1st day.....	140/90 mm. Hg	6th day.....	140/108 mm. Hg
2nd day.....	150/100 mm. Hg	7th day.....	152/110 mm. Hg
3rd day.....	130/106 mm. Hg	8th day.....	128/84 mm. Hg
4th day.....	132/108 mm. Hg	9th day.....	110/84 mm. Hg
5th day.....	144/114 mm. Hg	10th day.....	116/84 mm. Hg

A urinary concentration test was done on the twelfth day postpartum. Specific gravity ranged from 1.001 to 1.018. There was abundant volume in both the concentrated and dilute specimens. The Kahn test was done on the spinal fluid and was reported to be positive. Blood chemical determinations on the seventh and twelfth days postpartum showed creatinine, 1.3 mg. per cent; urea nitrogen, 13 and 10 mg. per cent respectively, and uric acid, 6 mg. per cent.

After discharge on the 13th day postpartum, the patient was referred to the Nephritis and Hypertension Clinic at the New York University College of Medicine. Arranged as Table I are significant laboratory findings obtained during her visits to the clinic.

TABLE I
Postpartum Observations in Nephritis and Hypertension Clinic at the New York University College of Medicine

Date	Blood pressure	Urine
6/26/40	134/90 to 160/100	Sp. gr., 1.010; protein, trace; red blood cells, 100 to 150
7/2/40	124/82	
8/13/40	114/80	Sp. gr., 1.016; protein, faint trace; no red blood cells
9/24/40	112/74	
10/15/40	126/80	Sp. gr., 1.020; no protein; no red blood cells
Nov., 1940	122/82	
Dec., 1940	130/90	No proteinuria or hematuria
Jan., 1941	120/80	
Feb., 1941	130/82	
Mar., 1941	114/78	
May, 1941	116/80	
June, 1941	130/86	
Oct., 1941	126/90	
Apr., 1942	120/80	
		Normal
		Normal

There were no specific complaints on any visit.

Renal function was investigated* by methods applied by Smith, Goldring and Chasis.¹⁵ Inulin clearance was used as a measure of the rate of glomerular filtration, diodrast clearance as a measure of the effective renal blood flow and diodrast Tm (tubular mass) to indicate the available functioning tubular tissue. At 5 months' gestation, which was 16 days after nephrectomy, the filtration rate and the effective renal blood flow were 75 per cent of normal, whereas the diodrast Tm was 50 per cent normal. (This was computed by comparison with average values in

* These tests were done, and will be reported in detail, by Drs. H. C. Taylor, Jr., Irwin Wellen and Catherine Welsh, of the Department of Obstetrics and Gynecology, New York University College of Medicine.

normal pregnant women.) These tests were repeated at 1, 5 and 9 months after delivery, the latter being 1 year after nephrectomy. The filtration rate was unchanged, the effective renal blood flow rose to normal level and the diodrast Tm increased to 80 per cent of the normal value. They represent, of course, the renal function of the remaining right kidney.

Pathologic Findings

The left kidney, operatively removed, appeared to be normal in size. The capsule stripped readily, leaving a somewhat pale, grayish pink surface. The organ was bisected in the longitudinal plane directed toward the pelvis. The cut surface revealed normal markings. The cortex was uniform in width and appearance. The pelvis was blood-stained and there were a few fresh clots adherent to the lining. On washing, tiny hemorrhages were visible in the pelvic lining and in the portion of the ureter attached to the pelvis.

Histologic Study. Blocks were fixed immediately in Helly's fluid, 10 per cent neutral formalin and Kopsch's fluid for mitochondria. Frozen sections were stained with Sudan IV for fat. Blocks were taken from all portions of the kidney and sections were cut in short strips composed of 16 to 20 serial sections, 5 μ in thickness. Alternating slides were stained by Masson's trichrome method (as modified by Goldner), and with azan-carmin. Some sections were stained by Mallory's method using phosphotungstic acid hematoxylin; others with van Gieson's picrofuchsin stain combined with Weigert's elastic tissue stain. Additional sections were stained for reticulum by the method of Foot and some were stained with Giemsa's stain. Tissue fixed for mitochondria was stained by the Altmann-Kull method.¹⁶

Sections from all parts of the kidney revealed uniform structure. The architecture was well preserved, but even at low magnification a striking alteration was visible in all the arterioles and a similar change could be traced backward in the medium-sized interlobular arteries. Except for the presence of a few red blood cells in some tubular lumina, no changes were found in the glomeruli or the tubules.

The vascular change consisted of hyperplasia and hypertrophy (Fig. 1) of the medial cells which thickened the walls of all the arterioles at the expense of the caliber of the lumina. In the terminal portions of the afferent arterioles this hyperplasia and hypertrophy produced small tumorlike swellings at their entrance into the glomeruli (Fig. 2). These correspond to the "Polkissen" which represent the normally thickened and expanded portion of the termination of the afferent arteriole as it enters the glomerulus. The hypertrophy of the terminal portions of the afferent arteriole in places indented the glomerulus. In other places it indented the neighboring tubules. The

lining endothelium appeared normal and there was no evidence of unusual distal extension or reduplication of the elastica in these arterioles.

Sections stained with hematoxylin and eosin gave only a slight suggestion of the degree of this change or of the swelling of the individual cells, but preparations stained by Masson's trichrome method or by azan-carmine revealed the details perfectly. They showed that none of the medial cells was fibrillar. With the azan-carmine stain they seemed quite clear, almost without cytoplasm, but under high magnification with slightly reduced illumination, a cytoplasmic ground substance could be seen.

In the intralobular arteries the media was similarly hypertrophied (Fig. 3), but there was also intimal and subintimal proliferation of tissue that was composed of young fibroblasts, smooth muscle cells and dense collagen. This proliferation served to narrow the lumen. There was no reduplication of the elastic membranes in these vessels. One interlobular artery contained a hyaline, sclerotic, intimal plaque which eccentrically narrowed the lumen. A small plaque was present in one interlobar branch, but most of the lobar branches of the renal arteries showed no sclerotic changes or intimal thickening.

Search for chromophilic granules like those that may be found in the terminal portion of the afferent arterioles of other mammalian kidneys was negative. Study of the efferent arteriole where it could be detected showed that similar swollen medial cells were present. These were seen to extend for only 15 to 20 μ along the efferent arterioles.

Sections of the pelvis and calyces revealed no inflammatory changes. In one section there was a small fresh hemorrhage without reaction just beneath the lining epithelium. In one place the epithelium had been freshly eroded. A similar process was seen in a ureteral section. In addition, where the lining cells were intact, marked swelling of the superficial cells was observed. The cytoplasm appeared vacuolated due to the presence of faintly basophilic mucoid material. However, this material did not give a positive stain for mucin.

Pathologic Diagnosis. Diffuse medial hyperplasia and hypertrophy of the afferent arterioles; focal fibromuscular intimal sclerosis with narrowing of interlobular renal arteries; multiple hemorrhagic erosions of the pelvis and ureter; hematuria.

DISCUSSION

The changes detected in the kidney are unique in our experience and their counterpart has not been found thus far in a search of the literature. Jores,¹⁷ in reviewing vascular disease, recognized the

occurrence of hypertrophy and hyperplasia of the media of small arteries. Recently, Castleman, Smithwick and Palmer¹⁸ have studied biopsy material obtained from the kidneys of patients with very early stages of "essential hypertension." These observers report medial hypertrophy and other changes in 16 cases so studied. The possibility exists, therefore, that the lesion described here may be related to changes encountered in hypertension.

In unpublished studies of renal arterioles in experimental and human hypertension, nothing equal to it has been found in normal or ischemic kidneys of man or of several mammals, with or without hypertension. Other surgically-removed human kidneys, from males and nonpregnant females, similarly fixed, have not yielded such pictures of medial hypertrophy. This would exclude the mode of removal or time and manner of fixation as having any influence. Other kidneys obtained from patients dying in pregnancy or a few days postpartum have been equally free from such changes, but in none was fixation as prompt.

Since the patient did not have hypertension before nephrectomy or for some time after it, the morphologic changes found in the afferent arterioles can hardly be related to the patient's blood pressure. However, considering their universality, it seems likely that the same changes were present in the surviving contralateral kidney. If so, the removal of one kidney may have contributed to a lowering of the total blood flow due to narrowing of the vascular bed and to a reduction of normally functioning kidney tissue and of antipressor substances possibly produced by parts of one or both kidneys. Estimations of the renal blood flow in the surviving kidney were below normal for at least 9 months after contralateral nephrectomy. Such an imbalance might have precipitated hypertension of the renal type. Against this interpretation is the long interval (3 months) that elapsed before the onset of hypertension. Furthermore, it was mild and lasted only 6 to 8 weeks altogether.

Even if both the hypertension and the eclampsia are not renal in origin, the vascular changes in the kidney are inescapable. Both their etiology and rôle are equally obscure.* The intimal sclerosis found in some of the larger intrarenal arteries must be regarded as coincidental, for areas supplied by nonsclerotic arteries have equal arteriolar changes.

* Sections from this kidney were sent to Dr. Goormaghtigh, who wrote, on learning that eclampsia and mild hypertension had appeared in this patient: "It confirms me in the belief that in most cases hypertrophy of the juxtaglomerular apparatus precedes hypertension or is present in the early stages of the latter; e.g., scarlet fever with glomerulonephritis, and eclamptic toxemia."

The clear appearance of the medial cells and the total absence of acidophilic fibrils suggested the possibility that these muscle cells were fixed in a state of relaxation or diminished tonus compared with fibrillar contracted muscle. That such relaxation may be due to a specific mechanism preventing tonic contractions is worthy of consideration. In this connection it may be recalled that Meigs¹⁹ has shown that when Zenker's fluid (a form of which was employed in this case) is applied to unstriated muscle it does not cause contraction and fixes the cells at their original length.

Can pregnancy be accountable for the vascular picture? Hormonal influences come to mind and the only suggestion we can offer is indirect. Since in pregnancy the smooth muscle of the uterus, its vessels and often the lower urinary tract²⁰ exhibit alterations characterized by hyperplasia and hypertrophy, is it possible that in the kidney reported here we have encountered a similar effect in the renal arteries and arterioles? The description of the changes in the myometrium taken from Keiffer²¹ by Reynolds²² is worth comparing with my findings:

"The nature of the process by which hypertrophy of uterine muscle takes place during pregnancy is not generally known, although it has been most carefully studied in the guinea pig and in the human. In both species, the cells appear to imbibe water at the time the uterus begins to undergo distention with the products of conception. At the same time innumerable centripetal myofibrils envelop the sarcoplasm superficially. As progressive hydration continues the cytoplasm becomes more and more transparent while the superficial portion remains chromophilic."

The relatively clear, swollen appearance of the medial cells in my case suggests that they are possibly "hydrated," as well as relaxed.

Detailed histologic observations of vascular alterations during pregnancy have been scanty. One report, also by Keiffer,²³ may be relevant to the problems raised by my case. In a paper entitled "*De l'existence d'une glande myométriale dans l'uterus humain*" he has recorded histologic findings in the myometrium which include detailed studies of the uterine vessels. Noting the need for prompt fixation by quickly acting fixatives, he studied uteri obtained by cesarean section at term or almost at term, as well as uteri obtained in earlier stages of pregnancy. With photomicrographs he illustrated changes which he believed began in the intermuscular tissue and affected the vascular walls as well. Some of the latter bear a striking resemblance to the changes reported in this paper. The affected cells are mononuclear and increased in size, have finely granular cytoplasm and undergo amitotic division. In the vascular walls Keiffer observed that the smooth muscle cells also increase in size, lose their oblong shape and are finally indistinguishable from the enlarged cells of connective

tissue origin. I have confirmed this appearance in Zenker-fixed fresh uteri (Fig. 4) furnished through the courtesy of Dr. William Studdiford. Like Gérard,²⁴ who found similar changes in the myometrium of the mouse and rat, Keiffer found much glycogen* in the swollen cells.

Changes like these in the rabbit had first led Ancel and Boin,^{25†} (1911) to propose the existence of a myometrial gland in the rabbit. Subsequently, Keiffer^{21, 23} had confirmed their findings in the guinea pig and human species. But he was not certain that there was sufficient evidence to establish the existence of a myometrial "gland," and preferred the term "myometrial placenta" for the changes until evidence of secretory activity is established.

In a later review of changes occurring in the walls of the uterine arteries after parturition in the guinea pig, Prenant²⁶ cited several reports of the occurrence, during pregnancy in man and in other species, of giant mononuclear cells in the media of these vessels. They have been ascribed to invading decidual cells,²⁶ to transformed medial muscle cells,²⁶ or to intimal cells.²⁷ Further investigation of the uterine arteries and veins and similar vessels in other organs during pregnancy seems indicated. I have examined the kidneys of five patients dying at term. No similar vascular changes were encountered, but in no case was fixation accomplished in less than 1 hour after death and there may have been postmortem alteration. The lack of material from cases of fatal eclampsia has precluded search for comparable changes in that group.

If the suggestion offered is valid, and the vascular change found in one kidney was reversible, an hypothesis based on the influence of pregnancy may be offered to account for (1) the occurrence of such obvious medial hypertrophy in this patient; (2) a progressive effect on the surviving kidney with (3) consequent development of altered function, resulting (4) in hypertension and possible eclampsia, and followed (5) by the involution of such changes and recession of the hypertension with the termination of pregnancy.

The unilateral hematuria of 3 years' duration is still unexplained. There was no calculus formation, neoplasm, or chronic inflammation. The erosions in the pelvis and ureter were fresh. If similar erosions were forming and healing during the 3 years prior to nephrectomy,

* Unfortunately, the kidney reported here was not fixed in absolute alcohol, which would have been ideal for demonstrating that glycogen might account for the clear appearance.

† Gérard²⁴ expressed the view that the so-called myometrial gland in the rat and mouse was not the same as that described in the rabbit by Ancel and Boin. Keiffer gives no explanation for equating these structures.

they might account for the hematuria but we have no proof of their occurrence. Very little blood was found in the tubules. If related to the vascular lesions, more erythrocytes might be anticipated in Bowman's spaces or in the tubules than were found.

The peculiar appearance of the lining cells of the pelvis is also unexplained. Modern textbooks of histology make no reference to this picture in human kidneys or ureters. However, in the older literature there are observations of cellular changes like these (see Petersen²⁸ for a discussion of its occurrence). However, no correlation with sex, age, or physiologic activity was established. The relationship to pregnancy is being explored separately.

It is to be emphasized that the patient's blood pressure was normal in the presence of these alterations. If the medial hypertrophy commonly observed in hypertension is comparable, then this case supports the conclusion that such hypertrophy may precede hypertension rather than be a consequence of it.

Finally it may be noted that the changes in the arterioles reported here extend uniformly along the vascular channels. The so-called "Polkissen" participate equally and there is no visible difference between the end-portions of the afferent arterioles and the proximal portions. This is true for deeply placed afferent arterioles as well as for the glomerular vessels near the capsule. The swollen appearance of the cells of the "Polkissen" suggests that they are derived from the same tissue and, at least under the conditions reported here, may have abundant cytoplasm resembling smooth muscle.

SUMMARY AND CONCLUSIONS

A case is reported of chronic unilateral hematuria in a colored female, 29 years old, who was cured by nephrectomy during the fifth month of pregnancy. The removed kidney revealed unusual, massive, medial hyperplasia and hypertrophy of the arterioles. There was accompanying intimal fibromuscular hyperplasia of the interlobular branches of the renal artery. Focal fresh hemorrhagic erosions were found in the pelvis and ureter.

The medial hypertrophy could not be accounted for by any known or demonstrable injury. An hypothetical explanation is offered, based on the theory that hormonal activity during pregnancy might be responsible for changes in the arteriolar smooth muscle comparable to those found in the gravid uterus.

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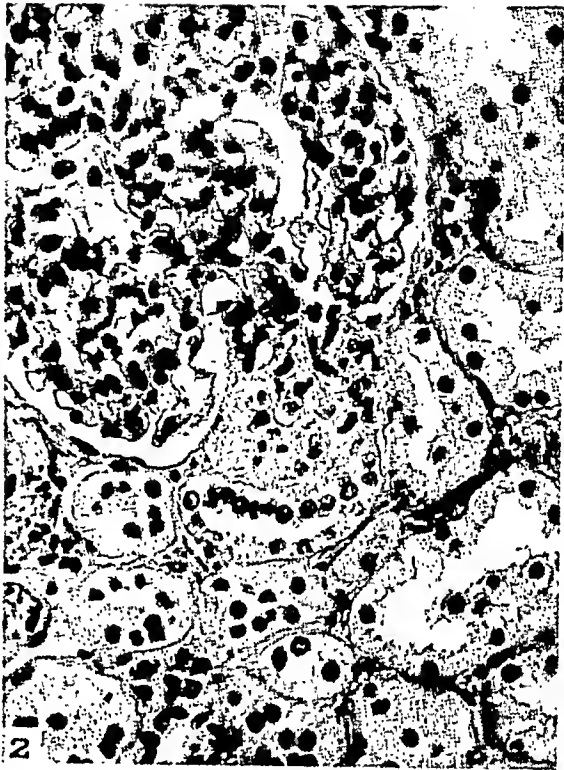
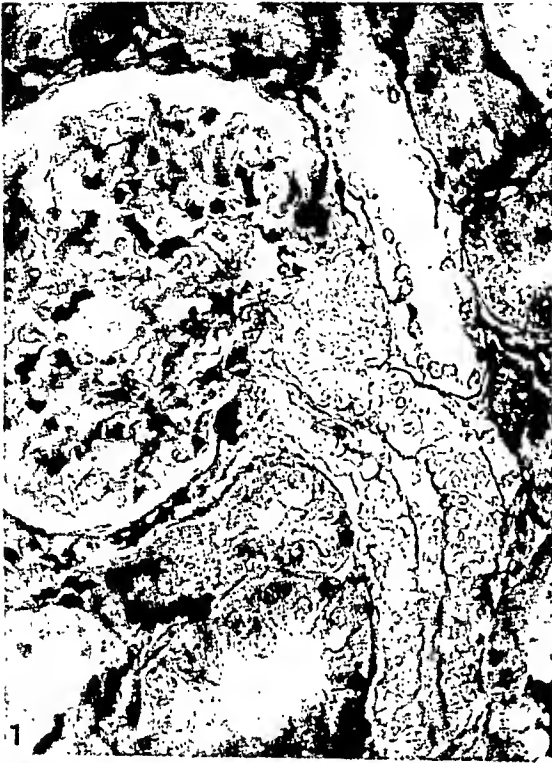
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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 17

- FIG. 1. Sagittal section through the terminal portion of an afferent arteriole and glomerulus showing hyperplasia and hypertrophy of the media due to afibrillar clear cells. There is an asymmetrical expansion of the juxtaglomerular portion. Azan-carmin stain. $\times 305$.
- FIG. 2. Transsection through the vascular pole of a glomerulus showing the hyperplastic "Polkissen" indenting the adjacent distal tubule. The latter exhibits palisading of the nuclei in the region of contact. This formation is designated as the macula densa. Hematoxylin and picro-fuchsin stain. $\times 320$.
- FIG. 3. A view of an intralobular renal artery exhibiting fibromuscular hypertrophy of the intima and media with narrowing of the lumen. Hematoxylin and picro-fuchsin stain. $\times 130$.
- FIG. 4. A view of several uterine arterioles of different size, in cross section, showing the medial hypertrophy occurring during pregnancy (uterine tissue obtained by hysterectomy during the 14th week of pregnancy; operation performed for delayed abortion in a psychotic patient of 25 years). Hematoxylin and picro-fuchsin stain. $\times 245$.



Graef

Medial Arteriolar Hypertrophy in Pregnancy



PATHOLOGIC CHANGES PRODUCED IN RABBITS BY A TOXIC SOMATIC ANTIGEN DERIVED FROM *EBERTHELLA TYPHOSA* *

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The isolation of purified antigenic materials from cultures of the *Salmonella* group of organisms by Boivin, Mesrobian and Mesrobian¹ and by Raistrick and Topley,² and the experiments of Henderson and Morgan³ with a protein-free antigen obtained from *Eberthella typhosa* have demonstrated that substances of relative purity and high antigenic and toxic activity may be isolated from organisms of the enteric group. In recent studies I have described the preparation of a somatic antigen from *E. typhosa* cultured in a synthetic medium⁴ and have presented an analysis of its toxic and immunologic properties.⁵⁻⁷ The possible rôle of this material in the clinical manifestations of typhoid fever was discussed.⁵ Because of its lethal effects on a variety of animals,⁵ its destructive action on leukocytes^{5, 6} and the inflammatory reaction that developed at the site of intradermal injection of a similar preparation employed in some earlier experiments,⁸ it was decided to investigate the local and general systemic effects of this material on tissues of rabbits treated for varying periods of time by several routes of injection. The present paper describes the results of these experiments.

MATERIALS AND METHODS

Preparation of Antigen

Following the technic previously described,^{4, 5} involving repeated precipitations with alcohol and resuspension in water of a material obtained from cultures of *E. typhosa* grown in a liquid medium containing only ingredients removable by dialysis,⁴ an antigenic material containing the Vi antigen was prepared which possessed all of the properties of the substance studied earlier.⁵

Methods of Administration

(a) *Intradermal.* Rabbits were given five intracutaneous injections of the antigen and these areas of skin were excised at intervals for microscopic examination. In Table I the details of this experiment are summarized.

(b) *Fixation Experiments.* Following the technic of Menkin,⁹ sev-

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eral rabbits received injections of the material to determine its efficacy in inducing inflammatory fixation.

(c) *Intravenous Injections.* Two groups of rabbits were given intravenous injections of various amounts of the material into the marginal vein of the ear at intervals of several hours. The first of these groups received injections over a much shorter period of time than did the second. Experimental details are given in Table II.

(d) *Intracardiac Injections.* To seven rabbits injections of antigen were administered into the left ventricle of the heart. This procedure was carried out in order to reduce, if possible, the pronounced injurious effects on the pulmonary tissue noted in the animals mentioned in (c), and to insure a maximum effect on the peripheral circulation. Table III summarizes the data.

Treatment of Tissues

Representative specimens of tissue were fixed in a 4 per cent solution of formaldehyde and in Zenker's fixative, and embedded in paraffin. Sections 7 μ in thickness were cut and stained with either hematoxylin and eosin or eosin and methylene blue.

The findings in any animals showing evidence of coccidiosis on gross or microscopic examination were excluded.

RESULTS

Experiment 1. Local Changes Following Intradermal Injection of the Antigen

The changes induced in the tissues of the skin after various intervals of time following the injection of antigen were studied. Two adult albino rabbits were shaved over their flanks and 1 mg. of the antigen was injected intradermally into each of five areas on each animal. Segments of skin including these areas were excised at intervals of 16 to 336 hours and from them sections were prepared as indicated in Table I.

Microscopic examination of the inoculated sites removed at the end of 16 and 40 hours revealed a marked inflammatory reaction characterized by hyperemia, edema and infiltration with polymorphonuclear leukocytes and deposition of considerable amounts of fibrin. At 96 hours many of the polymorphonuclear leukocytes showed evidence of necrosis whereas the hyperemia and edema had decreased. After 168 hours the centers of the lesions had become completely necrotic and only a few degenerated polymorphonuclear cells could be distinguished. At the periphery of the lesion, fibroblastic proliferation was evident which by 336 hours had formed a fibrous capsule.

Experiment 2. The Antigenic Material as an Agent in Inducing Inflammatory Fixation

Employing the technic of Menkin,⁹ two rabbits were given 5 mg. and two others 1 mg. of the material. The antigen in 1 cc. of physiologic saline solution was injected into the skin of the left foreleg; 1 cc. of physiologic saline solution was injected into the right foreleg as a control. After 18 hours, 1.5 cc. of 1 per cent trypan blue was injected into the hypodermis of each foreleg in the area previously

TABLE I
Results of Intradermal Injection of Antigen

Rabbit no.	Intradermal injection of antigen	Time after injection at which area was excised	Appearance of reaction
81 and 82	1 mg.	16 hours	Edematous, erythematous areas 2 cm. in diameter
		40 hours	Edema and erythema less intense
		4 days	Erythema absent and edema decreased; small nodules palpable in the center of the area
		7 days 2 weeks	Nodules have become more firm Small, firm, whitish intracutaneous nodules 0.75 cm. in diameter

treated. Each animal was sacrificed 3 hours after injection with dye and the lymphatics and lymph nodes draining the area were examined. The areas of skin into which the injections were made were excised and examined microscopically. At 18 hours, the areas injected with the antigen consisted of small (1 cm.) indurated lesions which exhibited central necrosis with surrounding zones of erythema. No significant changes were noted in the areas of the right legs injected with saline solution. On sacrificing the animal, the lymphatics of the right legs and the axillary lymph nodes were stained blue, indicating that free lymphatic drainage of the dye was present. The lymphatics and lymph nodes on the left side contained no dye. Thus inflammatory fixation depending upon lymphatic blockade had taken place.

Microscopic examination of the areas injected with the antigen showed edema and hyperemia with heavy leukocytic infiltration. The lymphatics frequently contained fibrinous thrombi and there was a network of fibrin in the interstitial spaces. Small blood vessels and capillaries were dilated and congested. In the lymph nodes the sinusoids were seen to be crowded with polymorphonuclear cells. Histologic examination of tissues from the control limbs revealed no significant abnormalities. The material under investigation therefore has the capacity to induce a marked inflammatory fixation.

Experiment 3. Pathologic Changes Induced in the Organs of Animals Following Intravascular Injections of the Antigen

These experiments may be divided into two groups, as the animals received somewhat different treatment.

The rabbits of group A were given intravenous injections over periods extending from 24 to 48 hours. At the end of various intervals, if the animals had not succumbed, they were killed by the intravenous injection of air and tissues were taken for section. The dosage and time intervals are given in Table II.

The animals in group B received intravenous and intracardiac injections over a more extended period of time. The intervals and dosage are summarized in Table III.

TABLE II
Results of Intravenous Injection of Antigen

Rabbit no.	Total amount of antigen injected	Number of injections	Number of days injected	Last dose of antigen	Manner and time of death in hours after last injection
24 and 25	0.5 mg.	1			1½ Killed with intravenous air
26 and 27	0.5 mg.	1			1½ Killed with intravenous air
29	0.5 mg.	1			2 Killed with intravenous air
30 and 31	0.5 mg.	1			5 Died of injection
34	0.5 mg.	1			10 Died of injection
32 and 33	0.5 mg.	1			24 Killed with intravenous air
1	1.0 mg.	2	2	0.5 mg.	2 Died of injection
12	11.0 mg.	2	2	6.0 mg.	1½ Died of injection
4 and 48	11.0 mg.	2	2	10.0 mg.	8 Died of injection
15	1.5 mg.	4	2	0.2 mg.	3 Died of injection
17	20.0 mg.	5	1	10.0 mg.	1½ Died of injection

TABLE III
Results of Intravenous and Intracardiac Injection of Antigen

	Rabbit no.	Total amount of antigen injected	Number of injections	Number of days injected	Last dose of antigen	Manner and time of death in hours after last injection
Intravenous injections	18	6.3 mg.	8	5	5 mg.	24 Killed with intravenous air
	19	15.7 mg.	11	5	5 mg.	1 Died of injection
	20	5.0 mg.	9	4	2 mg.	24 Killed with intravenous air
	21	16.0 mg.	10	5	5 mg.	9 Killed with intravenous air
	22	15.0 mg.	16	8	3 mg.	5½ Killed with intravenous air
	23	6.0 mg.	11	8	2 mg.	24 Killed with intravenous air
	124	12.0 mg.	2	2	10 mg.	10 days Killed with intravenous air
Intracardiac injections	40	0.25 mg.	3	3	0.1 mg.	24 Killed with intravenous air
	41	0.18 mg.	3	1	0.05 mg.	24 Killed with intravenous air
	42	0.40 mg.	5	3	0.20 mg.	6 Killed with intravenous air
	43	0.50 mg.	6	3	0.20 mg.	6 Killed with intravenous air
	44	5.68 mg.	9	4	2.5 mg.	2 Killed with intravenous air
	45	7.80 mg.	6	4	5.0 mg.	3 Died of injection
	46	12.70 mg.	6	4	5.0 mg.	2 Killed with intravenous air

Gross Examination

At autopsy there were no noteworthy changes in the majority of the organs of the animals of group A. Small areas of hemorrhage were observed in the lungs of some and petechial hemorrhages were noted in the small intestine. The normal intestinal contents were frequently replaced by mucus. In group B, in addition to the above changes, some of the livers showed small, white areas scattered over the surface and in some of the animals receiving intracardiac injections there was a fibrinous pericarditis.

Microscopic Examination

Heart. Group A. In occasional animals there were scattered, small areas in which the muscle fibers stained with eosin more intensely than normal. In the more markedly altered areas, necrosis of a few muscle fibers and leukocytic infiltration were noted.

Group B. Most of the specimens from animals in this group had changes similar to those described in group A. However, in a number of the animals that received large intracardiac injections there were larger areas showing degeneration of muscle fibers. Such cells had lost their striations and coagulative necrosis of the cytoplasm was evident. In two animals, nos. 23 (Fig. 1) and 124, living for longer intervals, the degenerative changes were accompanied by a proliferation of fibroblasts and beginning cicatrization. The sections from one rabbit showed an acute fibrinous pericarditis with underlying necrosis and inflammation of the muscle tissue. This lesion was undoubtedly the result of the escape of antigen into the pericardium at the time of injection.

Lung. Group A. The alveolar capillaries and blood vessels were engorged and contained a greatly increased number of polymorphonuclear leukocytes. In some specimens hemorrhage into the alveoli and patchy atelectasis were observed; other alveoli were filled with a serous precipitate. These changes were most marked in the animals given the larger dosage.

Group B. Areas of atelectasis and the accumulations of polymorphonuclear leukocytes in alveolar capillaries were also seen in this group. Some alveoli contained masses of polymorphonuclear leukocytes as well as serous precipitate. Thrombosis of small and medium-sized blood vessels was present in several animals (Fig. 2). Occasionally there was evidence of early stages of organization of the thrombi (Fig. 3). In two rabbits, nos. 22 and 24, focal areas of inflammatory consolidation were noted. These contained collections of mononuclear

leukocytes. The alterations in the lungs were less marked in the animals in which the antigen was injected into the left ventricle.

Liver. Group A. In the animals given the smaller dosage, scattered, isolated liver cells or small groups of cells exhibited hyalinization of the cytoplasm and pyknosis of the nuclei. Polymorphonuclear leukocytes had invaded or collected around the more extensively damaged cells. Following larger doses of antigen, definite areas of focal necrosis of hepatic cells were observed and many cells had entirely lost their structure. The blood vessels were engorged with erythrocytes.

Group B. The above-mentioned changes were better defined in the livers of this series of animals (Fig. 4). In occasional lesions, mononuclear phagocytes were present in the sinusoids about the areas of focal necrosis (Fig. 5). In animals nos. 44 and 45, which died soon after large, intracardiac injections of the antigen, extensive areas of necrosis of liver cells with few polymorphonuclear leukocytes in the sinusoids were observed. In addition to these changes, there were numerous occluding or mural thrombi in the central hepatic and larger veins (Fig. 6). These thrombi consisted of masses of an eosinophilic, hyaline material in which degenerating polymorphonuclear leukocytes were embedded and which were often surrounded by a network of fibrin.

Intestine. No constant pathologic changes were noted in the small intestines of either group. However, in some of the animals receiving large amounts of the antigen, there were indications of cellular necrosis and phagocytosis in the centers of some of the lymphoid follicles.

Spleen. The changes observed in the spleens of the animals in both groups were variable. Frequently the sinusoids, in rabbits killed after the longer intervals following the last injection, contained excessive accumulations of polymorphonuclear leukocytes. Occasionally thrombi were also observed in the vascular spaces. Phagocytosis of cells, cell fragments and an eosinophilic material by the mononuclear cells lining the sinusoids was apparent. In some of the specimens this phagocytic activity appeared considerably increased. Increased mitotic activity was also evident in some instances in the cells of the lymphoid follicles.

Adrenal. In both groups A and B, microscopic examination revealed varying degrees of degenerative changes in the cortical cells. In many there was evidence of injury or necrosis of isolated cortical cells. In the glands obtained from animals nos. 19 and 124 occasional small groups of cells had become necrotic and were invaded by polymorphonuclear leukocytes.

Kidney. The changes in these organs from both groups A and B

consisted mainly of congestion and hyperemia of the blood vessels. In rabbits nos. 19 and 20, thrombosis of the capillaries of the glomerular tufts and acute, tubular degeneration were noted.

Bone Marrow. Group A. In the animals dying within 1 to 3 hours after the last injection, the marrow was observed to contain relatively few adult polymorphonuclear cells. With longer periods following the final injection, the bone marrow contained large numbers of young polymorphonuclear leukocytes and myelocytes. In some animals there was an extravasation of blood into the marrow spaces.

Group B. The sections of the bone marrow were characterized by a marked hyperplasia of cells of the granulocytic series and alterations of the megakaryocytes marked by pyknosis of the nuclei and occasional invasion of the cell by polymorphonuclear leukocytes. Areas of necrosis and mononuclear cell infiltration were seen in the specimens from a few of the animals. Phagocytic cells were seen in many cases to have ingested fragments of the necrotic cells.

These changes, although variable, were more pronounced in the series of animals that received the antigen by the intracardiac route. Widespread necrosis of cells with or without hemorrhagic extravasation was most marked in the animals receiving the larger amounts of antigen shortly before death in whose bone marrow only a few mature leukocytes remained. There was hyperplasia of immature cells of the granulocytic series with increased mitotic activity which led them to overshadow the erythroblastic cells. Thrombi similar to those described in the liver and spleen were occasionally observed in the vascular sinuses of the marrow.

DISCUSSION

These experiments are of interest in that they indicate that the somatic antigen of *E. typhosa*, which is released on disintegration of the bacterial cell, has toxic effects on local injection and widespread destructive action when it is carried by the blood stream into various tissues. As previously reported,⁵ the animals give evidence of its toxic action on injection as indicated by labored respiration. Many also have a profuse diarrhea and their temperatures rise from 101° or 103° F. to levels as high as 105° to 107° F.

The congestion and hemorrhages in various organs as noted at autopsy have been described by Arima¹⁰ in animals treated with a toxic solution derived from cultures of the typhoid bacillus. Similar observations and necrosis of liver cells in rabbits were recorded by Spanedda¹¹ and Dennis¹² who used toxic substances prepared from *E. typhosa* by the method of Boivin.¹

The toxic action of the antigen on liver cells, lymphoid tissue, cardiac muscle and polymorphonuclear leukocytes is most prominent. From the evidence presented, it may be concluded that the destructive action of the antigen on leukocytes is not exerted solely against the polymorphonuclear leukocytes in the circulating blood as observed in previous experiments using rabbits⁵ and human beings,¹³ but that the substance reaching the bone marrow via the blood stream also destroys the cells *in situ*, producing areas of necrosis in some instances. Likewise the material has been demonstrated to destroy hepatic cells with a production of focal necroses. The lesions in the liver and bone marrow may, by their location, suggest some relationship to the focal necroses found in the bone marrow and liver of patients dying of typhoid infection as described by Mallory.¹⁴ It is perhaps noteworthy that these lesions in the human material occur without the presence of demonstrable typhoid bacilli in the sections, as pointed out by Mallory¹⁴ and Goodpasture.¹⁵ Goodpasture has postulated that these focal necroses indicate the action of an endotoxin in the circulating blood derived from phagocytosed and lysed bacilli. Thus the powerful tissue toxicity of the antigen used in these experiments may fulfill this rôle since its toxicity for these tissues has been demonstrated and it is released on the disintegration of typhoid organisms.

The occurrence of thrombi in the vessels of the lung, liver, spleen and in the sinusoids of the liver, spleen and bone marrow gives evidence that this material injures vascular endothelium leading to thrombosis. This action may possibly be related to the mechanism responsible for the widespread thrombosis of blood vessels in the tissues of patients dying of typhoid fever,¹⁴ though the collections of large mononuclear cells within the vessels and beneath the endothelium as described by Mallory¹⁴ were not reproduced in these experimental animals.

Thus, although the lesions in the tissues of these rabbits injected with the toxic antigen derived from *E. typhosa* have in many instances shown no striking cytologic similarity to the lesions observed in tissues of fatal cases of typhoid fever in man, the production of lesions in the liver, spleen, bone marrow and blood vessels indicates that this potent tissue toxin may play a rôle in the production of changes observed in the tissues of patients dying of typhoid fever.

SUMMARY

1. The toxic somatic antigen isolated from *E. typhosa* produces a marked inflammatory reaction when injected into the skin of a rabbit that leads to fixation *in situ* of an injected colloidal dye. The

resultant changes proceed to eventual necrosis and walling off of the area by fibrous tissue.

2. The antigen administered by intravascular routes causes widespread injury to capillary and blood vessel walls with subsequent thrombosis, and leads to the necrosis of cells of the liver, heart muscle, adrenal glands and bone marrow. The kidneys and the lymphoid tissue of the spleen and small intestine may also be injured. The injurious action of the antigen is followed by a hyperplasia of the bone marrow chiefly involving the granulocytic series of cells.

I wish to acknowledge with gratitude the advice and constructive criticism of Dr. G. A. Bennett of the Department of Pathology of the Harvard Medical School. The experiments on inflammatory fixation were carried out by Dr. Valy Menkin of the Department of Pathology. The author wishes to thank Dr. J. A. Horneff for assistance in the preparation of the photomicrographs.

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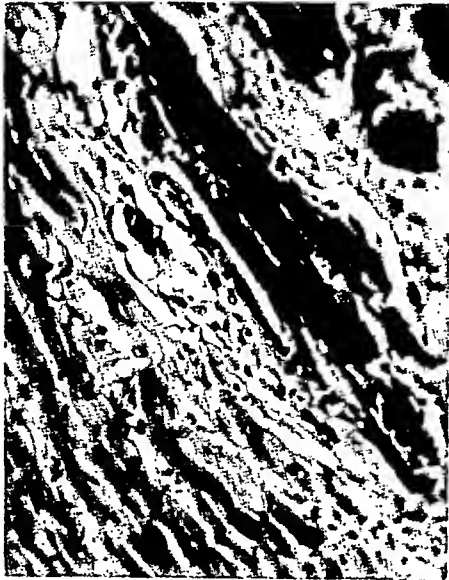
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DESCRIPTION OF PLATE

PLATE 18

- FIG. 1. Cardiac muscle from rabbit no. 23. Degeneration of cardiac muscle fibers with beginning cicatrization is present. Hematoxylin and eosin stain. $\times 200$.
- FIG. 2. Lung of rabbit no. 45. Thrombosis of a medium-sized vein and large numbers of polymorphonuclear leukocytes within the alveolar walls are notable. Eosin and methylene blue stain. $\times 200$.
- FIG. 3. Lung of rabbit no. 18. The thrombus in this small blood vessel is observed in an early stage of organization with a layer of endothelium extending over its surface. Hematoxylin and eosin stain. $\times 200$.
- FIG. 4. Liver of rabbit no. 20. Liver cells in this area have undergone degenerative changes and invasion by polymorphonuclear leukocytes. Hematoxylin and eosin stain. $\times 200$.
- FIG. 5. Liver of rabbit no. 18. In this area of necrosis there is complete loss of the liver parenchyma and infiltration by mononuclear cells. Hematoxylin and eosin stain. $\times 200$.
- FIG. 6. Liver of rabbit no. 44. A hyaline thrombus lies within the lumen of this hepatic vessel. Eosin and methylene blue stain. $\times 200$.

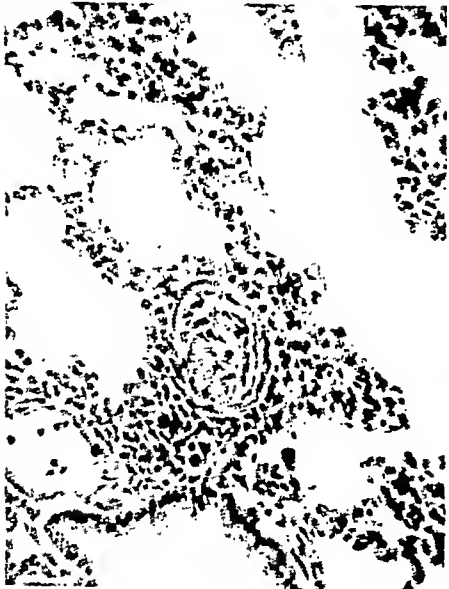
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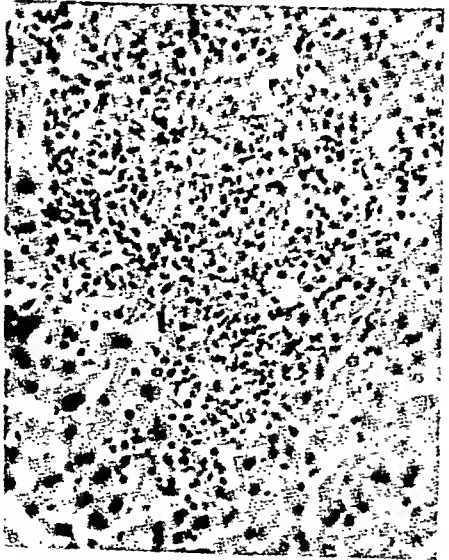
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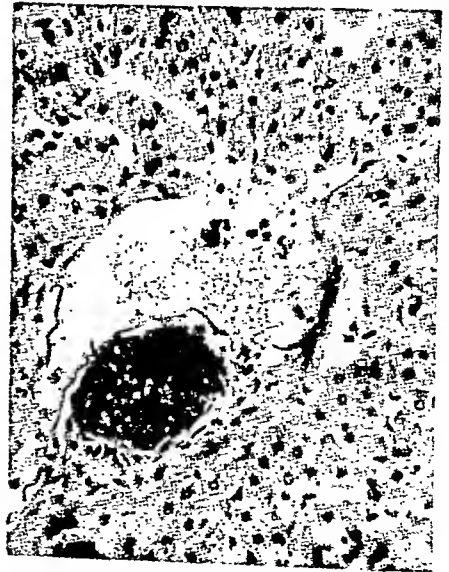
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6



EXPERIMENTAL NECROTIZING ARTERITIS IN DOGS

III. BILATERAL NEPHRECTOMY AS EFFECTIVE AS HEAVY METAL INJURY IN ITS PRODUCTION *

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In a recent publication I¹ described acute necrotizing arterial lesions which appeared unexpectedly during the course of experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level. Five dogs, maintained on a standard low protein diet, were made hyperproteinemic by repeated daily injections of plasma obtained from healthy donor dogs. Each then received a single subcutaneous injection of uranium nitrate; 5.0 mg. per Kg. Four of these showed acute necrotizing arterial lesions when they died 8 to 17 days later and the fifth dog showed healed lesions in the pulmonary artery when it was sacrificed 11 months later. Four additional dogs, maintained on the same standard diet, were made hypoproteinemic by repeated plasmapheresis, then injected subcutaneously with uranium nitrate. The two dogs which received 3.0 and 5.0 mg. per Kg. showed similar arterial lesions when they died 15 days after injection of the heavy metal, while the two dogs which received 2.0 and 2.5 mg. per Kg. failed to develop any arterial lesions.

These arterial lesions affected principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries), but were also found in other arteries (mesenteric, femoral, subclavian). They were consistently absent from the vessels of organs other than the heart and lungs.

Essentially, the lesion is an acute necrotizing arteritis apparently starting, and usually most marked, in the intima but not infrequently involving all three coats. In a few instances the lesion was situated primarily in the adventitia. Edema, together with swelling and fragmentation of collagen, seemed to be the initial change. In rapid succession, necrosis of collagen, outpouring of fibrin, massive polymorphonuclear reaction and disintegration of elastic tissue and the other elements in the arterial wall ensued. The process appeared to start in the connective tissue beneath intact endothelium, but in the advanced lesions ulceration with thrombosis was observed. Sometimes calcium was deposited in the necrotic lesions. Some of the lesions

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resembled those of periarteritis nodosa and rheumatic arteritis, but no claim of identity was made.

In a more recent article,² identical lesions in the same locations have been reported following the use of mercuric chloride instead of uranium nitrate. Two dogs, maintained on the same standard diet, were made hyperproteinemic by repeated daily injections of plasma obtained from healthy donor dogs. Each then received a single intravenous injection of mercuric chloride; 3.0 mg. per Kg. Both dogs showed acute necrotizing arterial lesions when they died 6 and 7 days after injection of the heavy metal. One of these dogs had a saccular aneurysm about 8 mm. in diameter situated on the anterior surface of the innominate artery immediately at its point of origin from the arch of the aorta.

Thus these experimental necrotizing arterial lesions have been produced with regularity by paying attention to three factors:

1. Standard low protein diet.
2. Plasma alteration (usually repeated intravenous injections of plasma obtained from healthy donor dogs).
3. Heavy metal injury (both uranium nitrate¹ and mercuric chloride²). Control observations indicated that the lesions were related to the experimental procedure and were not due to accidental or coincidental infection. Although some conjectures about the pathogenesis of the lesions have been made,¹ no satisfactory explanation for them has been advanced.

The present report attempts to elucidate one of the three factors, namely, heavy metal injury. Since both uranium nitrate and mercuric chloride produce renal injury with death in "uremia," the question naturally arose whether the arterial lesions are related to the heavy metal as such or whether they are due to the renal insufficiency which follows the injection of the heavy metal. To bring evidence to bear on this point it was decided to substitute bilateral nephrectomy for heavy metal injury. At the outset it was realized that the dogs might not live long enough to decide the question, but since the lesions had been observed as early as 6 days after mercuric chloride and since survivals of longer intervals after bilateral nephrectomy had been reported,³ the attempt was made and has been rewarded by finding less marked lesions, presumably in the early stages of their development.

METHODS

The methods have been described in detail in previous publications.^{1, 2} The three young, adult, female dogs used in the present studies were maintained on the standard diet which consisted of:

calves' liver (raw wet weight), 32 parts; cane sugar, 25 parts; corn-starch, 25 parts; butter, 12 parts; cod liver oil, 6 parts. Enough tomato juice was added to make a paste, of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per Kg. per day. One gm. of the McCollum-Simmonds salt mixture⁴ and 5 gm. of kaolin were thoroughly mixed with each day's diet.

The plasma injections, repeated six times per week for 3 to 4 weeks, averaged 102 cc. This amounted to one-fourth to one-third of the total quantity of circulating blood plasma in the recipient, and during the course of 3 to 4 weeks the total citrated plasma injected amounted roughly to five to six times the total volume of circulating blood plasma of the recipient. Each injection contained about 2.5 cc. of a saturated aqueous solution of trisodium citrate.

Nembutal anesthesia (40 mg. per Kg. in about 10 cc. of sterile distilled water injected intraperitoneally) was employed for the nephrectomy operations. In dog no. 40-52 the left kidney was removed 4 months before starting the plasma injections. The right kidney of dog no. 40-52 and both kidneys of the other two dogs were removed on the day following the last injection of plasma. The incision was paracostal in the anterior axillary line and the chief precaution taken in removal of the kidneys was the careful stripping of the perirenal tissues so as to expose the pedicle (artery, vein and ureter). This was tied with number 9 white silk thread that had been doubled twice to form four strands. No dressing was necessary. Sterile technic was observed throughout.

Necropsy was performed promptly after death of the dogs on the fifth or sixth day after removal of the kidneys. Blocks from all of the organs and from most of the tissues were fixed in a 4 per cent solution of formaldehyde and in Zenker's solution. Routine sections, made from the Zenker-fixed material embedded in paraffin and cut at 7 μ , were stained with hematoxylin and eosin. Special stains used in selected cases included: Verhoeff's stain for elastic tissue, sometimes combined with van Gieson's picro-acid fuchsin solution; von Kossa's silver nitrate method for calcium, scarlet red stain for fats; phloxine-methylene blue and Gram's stain for bacteria; and Levaditi's stain for spirochetes.

EXPERIMENTAL OBSERVATIONS

Table I gives in summary the experimental data and in Table II the size and anatomical distribution of the lesions are indicated.

Typical lesions are illustrated in Figures 1, 2, 4 and 7. Qualitatively the lesions were identical with those previously reported following the

use of uranium nitrate¹ and mercuric chloride.² Leukocytic infiltration with nuclear fragmentation is definite in all of these figures. While most of the leukocytes are most accurately described as "cells with distorted nuclei," some of them are recognizable as polymorphonuclear leukocytes. A rare mononuclear cell with faintly eosinophilic cytoplasm—presumably an eosinophilic myelocyte—has been observed,

TABLE I
*Summary of Experimental Data on Dogs with Bilateral Nephrectomy**

Dog number	Number of injections†	Total amount of plasma injected	Plasma protein concentration		Body weight‡	Height of non-protein nitrogen	Survival interval¶
			Before injection	After injection			
		(cc.)	(gm. per 100 cc.)	(gm. per 100 cc.)	(Kg.)	(mg. per 100 cc.)	(days)
40-52	24	2515	7.7	10.2	7.3	710	5
40-77	24	2515	7.1	9.3	5.2	295§	6
42-1	19	1825	5.9	8.3	5.6	538	6

* All dogs maintained on basal diet throughout entire period.

† The total period in which these injections were made was 3 to 4 weeks.

‡ Body weight at end of period of plasma injections.

§ This determination was made 2 days before this dog died.

¶ Interval between bilateral nephrectomy and death.

TABLE II
*Size and Distribution of Arterial Lesions**

Dog number	Ascending aorta	Left auricle	Pulmonary artery	Other arteries
40-52	++†	+		
40-77	+	++†	+	Myocardial arteriole, ++
42-1	+++†	++	+	Innominate, ++; arterioles in myocardium, ++ and in submucosa of stomach, +.

* The lesions have been graded as follows: ++ = gross lesion less than 1 cm. in maximum diameter; + = lesion discovered in microscopic section.

† Microscopic illustration.

but eosinophils were not a conspicuous part of the reaction. Edema with swelling and early fragmentation of collagen can be seen about the margins of some of the lesions (Figs. 2 and 4). Verhoeff's stains showed definite fragmentation of elastic tissue in some of the lesions (Fig. 5). No calcification nor thrombosis was seen in these early lesions. Frozen sections stained for fat have been negative.

Quantitatively the lesions were definitely smaller than those that have previously been reported.^{1, 2} In all probability they would have been overlooked in a routine necropsy. Careful examination of the areas in which the lesions were known to occur most frequently (pulmonary artery, endocardium of the left auricle, ascending limb of

the arch of the aorta including the mouth of the innominate artery) revealed a grossly positive result in only one dog. In the other two dogs there was questionable roughening of the intimal or endocardial surface in one or more foci that proved positive on histological examination.

As in previous experiments no organisms have been cultured from the blood during life or from the lesions at the time of necropsy. Nor have any organisms been demonstrated in or about the lesions with methylene blue, Gram's or Levaditi's stains. Evidence that sodium citrate, the anticoagulant injected with the donor's plasma, was not responsible for the development of the lesions has recently been published.⁵ Thus all control data continue to indicate that the lesions are related to the experimental procedure and are not due to accidental or coincidental infection.

DISCUSSION

From the results reported above, I believe the statement is justified that bilateral nephrectomy is as effective as heavy metal injury in the production of these necrotizing arterial lesions. The negative gross results in two of the dogs are probably related to the fact that the animals did not survive the operation for a sufficient period of time. In the positive animals the lesions are similar in all qualitative respects. Thus the question of heavy metal as such has been eliminated and the essential factor appears to be renal insufficiency.

The similarity of some of the lesions to those of periarteritis nodosa is evident from Figure 6 and has been commented on previously.¹ In this connection it is of interest that many human cases of periarteritis nodosa are preceded by diffuse glomerulonephritis.⁶ A survey of the 20 cases of periarteritis nodosa in the files of the Department of Pathology of Columbia University substantiates this view. In 12 of the 20 cases there was definite evidence of nitrogen retention and in the majority it was evident that the renal insufficiency antedated the arterial changes.*

The way in which renal insufficiency operates in the production of arterial lesions has interested clinicians since the time of Hippocrates and especially since the time of Richard Bright. Considerable impetus has recently been added to the problem by the work of Goldblatt in which he has shown that renal ischemia of moderate degree leads to hypertension, while renal ischemia severe enough to produce insufficiency with nitrogen retention leads to hypertension and arteriolar necrosis.⁷ Neither the pressor substance nor the necrotizing factor

* The author is indebted to Dr. J. W. Jobling for access to these files.

has been isolated but the latter appears to be definitely correlated with renal insufficiency. Winternitz and Katzenstein⁸ have reached the same conclusion regarding the rôle of renal insufficiency in the necrotizing arterial lesions which they produced in dogs by subtotal ureteral obstruction.

Concerning the manner in which renal insufficiency might operate in the pathogenesis of these arterial lesions, three possibilities come to mind:

1. Toxic factor—failure to eliminate something toxic to the arterial wall.
2. Nutritive factor—removal of something necessary for continued arterial integrity.
3. Imbalance—indirectly upsetting a balance necessary for arterial integrity. No evidence is available to substantiate any of these general possibilities.

The rôle of one of the other two factors—plasma alteration—is discussed in the following paper.

SUMMARY

1. Acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries) have been produced with regularity in dogs by combining three factors:
 - a. Maintenance on a standard low protein diet.
 - b. Plasma alteration (usually repeated daily injections of plasma obtained from healthy donor dogs).
 - c. Heavy metal injury—both uranium nitrate¹ and mercuric chloride.²
2. In the present report it has been shown that bilateral nephrectomy can be substituted for heavy metal injury, thus indicating that renal injury rather than heavy metal as such is the essential factor.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

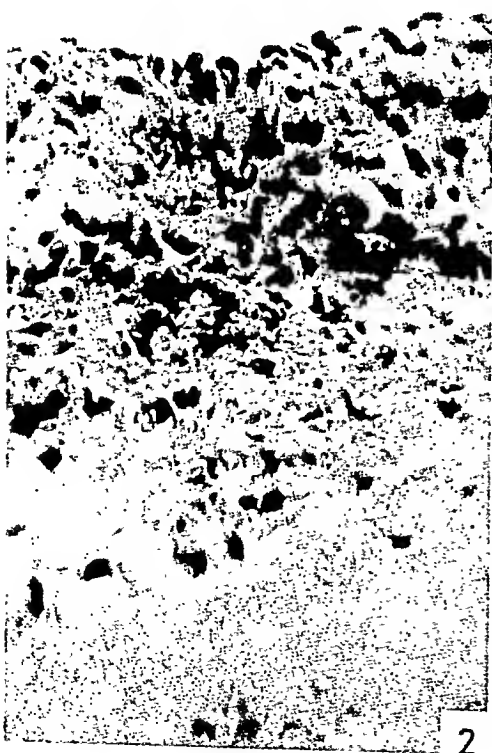
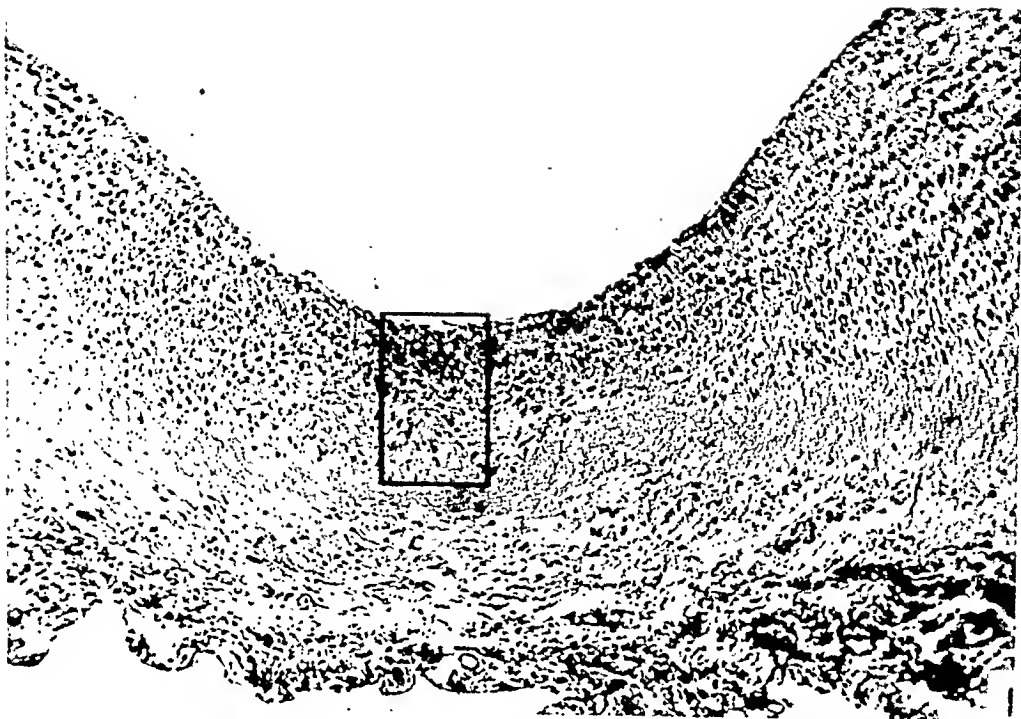
PLATE 19

Necrotizing arterial lesions in dogs. All sections were taken from dog no. 42-1 and were stained with hematoxylin and eosin.

FIG. 1. Acute necrotizing arteritis at point of origin of innominate artery from aorta. $\times 85$.

FIG. 2. (From area indicated in Fig. 1.) $\times 380$.

FIG. 3. Right ventricle. Necrotizing arteriolitis. $\times 380$.



Holman

Experimental Necrotizing Arteritis. III.

PLATE 20

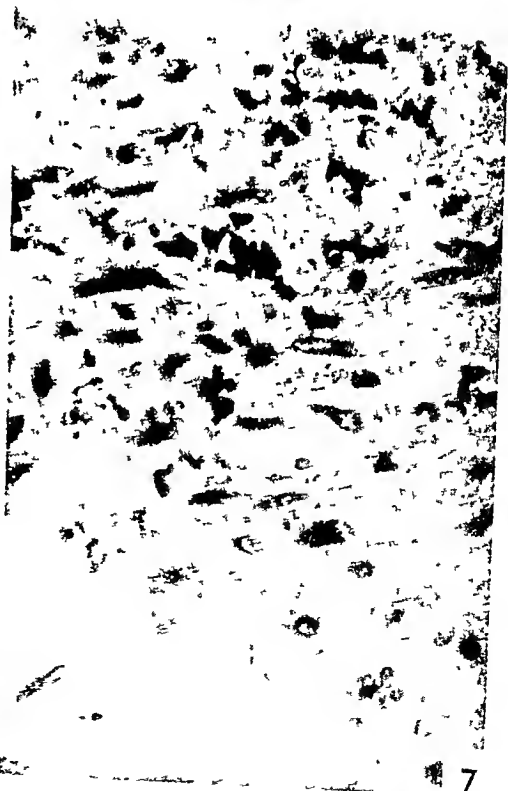
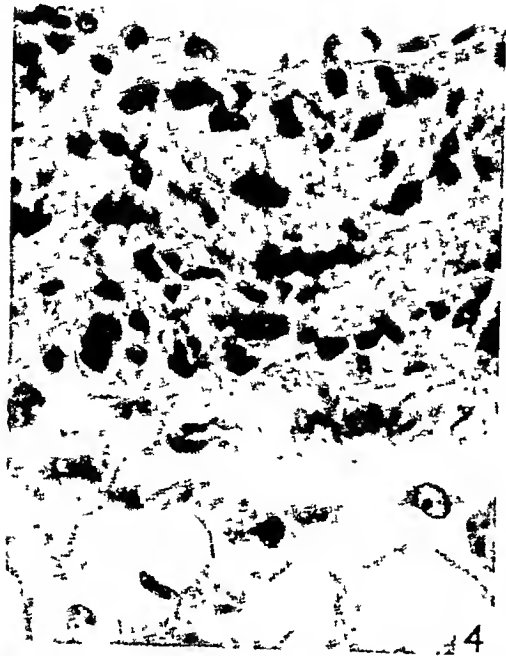
Necrotizing arterial lesions in dogs. All sections, except that used for Figure 5, were stained with hematoxylin and eosin.

FIG. 4. Dog no. 40-77. Acute necrotizing left auriculitis. $\times 520$.

FIG. 5. Dog no. 40-77. Acute necrotizing left auriculitis. Swelling and fragmentation of elastic tissue. Verhoeff's elastic tissue stain. $\times 350$.

FIG. 6. Dog no. 40-77. Left ventricle. Necrotizing arteriolitis simulating periarteritis nodosa. $\times 95$.

FIG. 7. Dog. no. 40-52. Acute necrotizing aortitis. $\times 520$.



Holman

Experimental Necrotizing Arteritis. III.



EXPERIMENTAL NECROTIZING ARTERITIS IN DOGS

IV. ALTERATION OF THE BLOOD PLASMA PROTEINS NOT ESSENTIAL *

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In previous publications ¹⁻³ evidence has been presented indicating that acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries) can be produced with regularity in dogs by controlling three factors:

1. Standard low protein diet.
2. Plasma alteration (usually repeated intravenous injections of citrated plasma obtained from healthy donor dogs).
3. Renal insufficiency produced by toxic doses of uranium nitrate,¹ or mercuric chloride,² or by bilateral nephrectomy.³

All of the control data have indicated that the lesions are related to the experimental procedure and are not due to accidental or coincidental infection.

In this paper data are presented which eliminate from an essential rôle one of the most puzzling of these three factors, namely, plasma alteration. If these observations are confirmed, the problem is reduced to two factors: (1) a dietary factor and (2) a factor associated with renal insufficiency.

During the course of experiments by Donnelly and Holman,⁴ designed originally to determine whether sodium citrate (the anti-coagulant used in obtaining plasma from the donor dogs) played any pathogenic rôle in these arterial lesions, 3 of 66 dogs receiving "citrate and saline" instead of "citrate and plasma" showed typical necrotizing arterial lesions when they died 10 to 14 days after a lethal dose of uranium nitrate or mercuric chloride. These were the first examples of necrotizing arterial lesions that had been encountered in a dog not maintained on the standard diet and not subjected to "plasma alteration."

About this same time Dr. M. C. Winternitz delivered a lecture at Duke University Medical School in which he presented illustrations of "hemorrhagic" and "necrotic" lesions in various organs and tissues of dogs that had been subjected to subtotal ureteral obstruction. Some of these lesions involved arteries and arterioles and resembled the

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necrotizing lesions that we had been obtaining. No mention was made of the diet on which his animals were maintained, and no "plasma alteration" was involved in his experiments. Some of the data which he presented at that time have since been published.^{5, 6}

These observations, plus the disconcerting fact that lesions had been observed in hypoproteinemic as well as hyperproteinemic dogs,¹ prompted the following experiments using *prolonged maintenance on the standard diet* before producing renal injury.

TABLE I
Summary of Experiments without "Plasma Alteration"

Dog number	Period on standard diet*	Type of renal injury	Maximum nonprotein nitrogen	Survival interval†
	(months)		(mg. per 100 cc.)	(days)
40-56	5	Previous left nephrectomy, right nephrectomy	20	0
40-83	5	Bilateral nephrectomy	348	3
40-85	5½	Bilateral nephrectomy	308	4
40-84	5	Bilateral nephrectomy	244‡	5
40-79	4	Previous left nephrectomy, uranium nitrate, 5.0 mg./Kg.	544‡	7
40-68	3	Uranium nitrate, 5.0 mg./Kg.	162§	35 (sacrificed)

* Period before renal injury was produced.

† Interval between renal injury and death.

‡ Last sample obtained on day before death.

§ Seven days after renal injury. Terminal nonprotein nitrogen, 44 mg./100 cc.

METHODS

The methods were the same as those outlined in the preceding paper.

EXPERIMENTAL OBSERVATIONS

Table I gives in summary the experimental data on the six dogs maintained on the standard diet for 3 to 5½ months before being subjected to interference with renal function. This consisted of bilateral nephrectomy in four of the dogs and of uranium nitrate injury (5.0 mg. per Kg. injected subcutaneously) in the other two dogs. One of the latter, dog no. 40-79, and one of the former, dog no. 40-56, had had the left kidney removed several months before commencement of the experiments with standard diet feeding. Four of the dogs died in "uremia" 3 to 7 days after the renal injury; one, dog no. 40-68, survived the renal injury and was sacrificed 35 days later; the other died of an operative accident. This last dog, no. 40-56, served as a control on diet alone. In this connection many of the dogs used in the studies on plasma protein regeneration at the University of Rochester have been maintained on the same standard diet for periods

of over 1 year and have not shown necrotizing arterial lesions.⁷ The only feature of note in the kidneys excised after several months on the standard diet was the presence of considerable fat, readily stainable by scarlet red, in the loops of Henle.

The size and distribution of the arterial lesions are indicated in Table II. No gross lesions were seen in dogs nos. 40-83 and 40-85. In dog no. 40-85 early lesions were present in the microscopical sections while in dog no. 40-83, which survived bilateral nephrectomy

TABLE II
*Size and Distribution of Arterial Lesions**

Dog number	Ascending aorta	Left auricle	Pulmonary artery	Other arteries, and remarks
40-56				Control dog; no arterial lesions
40-83		±	±	
40-85	+	+	+	
40-84	++	+++†	+++†	Innominate +++ (aneurysmal bulge)
40-79	++	+++†	+++†	Innominate +++ (aneurysm); common carotid +++†; subclavian +-; internal mammary ++; femoral ++; mesenteric +
40-68	++	+++	+++†	Artery in lung ++†; all lesions healing

* The lesions have been graded as follows: +++ = gross lesions over 1 cm. in maximum diameter; ++ = gross lesions less than 1 cm. in maximum diameter; + = lesions discovered in microscopical sections; ± = equivocal lesions.

† Microscopical illustration.

only 3 days, suggestive changes (edema, swelling of collagen, early pyknosis of nuclei and questionable leukocytic infiltration) were seen on histological examination, but no definite necrosis was present. In the other three dogs (nos. 40-84, 40-79, and 40-68) typical gross and microscopical lesions were found in the usual locations (Figs. 1-7; see preceding paper for description of lesions).

It is noteworthy that the findings in the nephrectomized dogs were directly proportional to the length of time the animals survived the operation. One (dog no. 40-56) died of an operative accident; one (dog no. 40-83, 3 days) was grossly negative and showed only suggestive changes on histological study; one (dog no. 40-85, 4 days) was grossly negative but showed definite microscopical lesions; and one (dog no. 40-84, 5 days) showed typical gross and microscopical lesions including an aneurysmal bulge on the anterior surface of the innominate artery at its point of origin from the arch of the aorta. Previously, gross lesions have not been observed earlier than 6 days after the production of renal insufficiency.

Two features of the lesions in dog no. 40-79 deserve special comment. First, there was a saccular aneurysm 9 mm. in diameter and partially filled with laminated thrombus on the anterior surface of

the innominate artery at its point of origin from the aorta. This is the second such aneurysm that has been found and both occurred at the same site (see dog no. 40-80² for the other). The second feature of interest is that the lesions differ to some extent from the ones usually seen. Instead of massive leukocytic infiltration and necrosis with partial liquefaction of the elements of the arterial wall, the lesion was chiefly a change in the elastic tissue. Grossly the affected vessels were crumpled and inelastic. Microscopically, instead of being undulating the internal elastic membrane was in a straight line (Fig. 5). Necrosis was obvious from the loss of nuclei and the more intense eosin staining of the smooth muscle cells, but leukocytic infiltration was minimal. These lesions resembled those previously described and illustrated in dog no. 38-24,¹ except that calcium deposition was much more conspicuous in dog no. 38-24. Possibly this was related to the time factor; dog no. 40-79 lived only 7 days after the renal injury while dog no. 38-24 survived 15 days. Renal injury in both was produced by uranium nitrate.

All of the lesions in dog no. 40-68 were healing or healed. This dog survived the acute phase of the experiment and was sacrificed with ether 35 days after the administration of uranium nitrate. This is the second dog that has shown healed lesions (compare dog no. 39-28 that was sacrificed 11 months after the administration of 5.0 mg. of uranium nitrate per Kg.¹). In dog no. 39-28 there was a complication—the presence of five adult female heart worms, *Dirofilaria immitis*, in the pulmonary artery, the vessel that showed the most marked healed lesions. This made the interpretation of the healed lesions difficult. In dog no. 40-68 no “heart worms” were present yet the lesions were similar in all respects except those related to differences in survival time.

DISCUSSION

The chief significance of these experiments is that they eliminate from an essential rôle one of the most puzzling features connected with these studies on “experimental necrotizing arteritis in dogs,” namely, “plasma alteration.” The original conjecture about the pathogenesis of these arterial lesions, “that they may be due to a hypersensitivity to some constituent of the blood of one or more of the donor dogs,” has to be modified. The problem apparently becomes one of disturbed metabolism and resolves itself into two factors: (1) a dietary factor and (2) a factor associated with renal insufficiency.

This concept of the problem brings these studies into closer correlation with those of Winternitz and his co-workers,^{5, 6} and with those

of Goldblatt⁸ and others. The factor common to all of this experimental work is renal insufficiency, a factor long suspected of playing a part in arterial disease in humans. Brief evidence for a possible etiological relationship between renal injury and periarteritis nodosa has been given in the preceding paper.

At the present time it seems to make little difference as to how the renal insufficiency is produced; *i.e.*, by heavy metals, by constricting the renal arteries, by subtotal ureteral obstruction, or by extirpating the kidneys. With constriction of the renal artery, hypertension ensues, and this may be responsible for a necrotizing process affecting the arterioles rather than the larger arteries. Winternitz and Katzenstein⁶ failed to observe any correlation between blood pressure and the anatomical lesions in their dogs, which involved arteries as well as arterioles. The few scattered determinations of blood pressure which I have made have not yielded any definite results.

Neither Goldblatt⁸ nor Winternitz and his co-workers^{5, 6} have found it necessary to control the diet of their animals. In my experiments to date, diet seems to play a definite rôle. Evidence for a dietary factor is admittedly incomplete but is accumulating and will be presented in the near future.

SUMMARY

1. In all of my previous publications on "experimental necrotizing arteritis in dogs,"¹⁻³ three factors have been constant:
 - a. Maintenance on a standard low protein diet during the course of the experiment (3 to 6 weeks).
 - b. Alteration of the blood plasma proteins—hyperproteinemia in 11 dogs by repeated injections of plasma obtained from healthy donor dogs; hypoproteinemia by plasmapheresis in 2 dogs.
 - c. Renal insufficiency—uranium nitrate in 8 dogs;¹ mercuric chloride in 2 dogs;² bilateral nephrectomy in 3 dogs.³
2. In the present report none of the dogs was subjected to any alteration of the blood plasma proteins but there was prolonged maintenance on the standard diet before renal injury was produced. Four of 5 dogs, maintained on the standard diet for 3 to 5 months and then subjected to renal injury (2 by uranium nitrate and 2 by bilateral nephrectomy), showed typical necrotizing arterial lesions when they died or were sacrificed 4 to 35 days later. The fifth dog, which survived bilateral nephrectomy only 3 days, showed suggestive early changes but no definite necrosis.
3. Prolonged maintenance on the standard diet is as effective as less prolonged maintenance on the diet *plus* "plasma alteration."

4. These experiments eliminate from an essential rôle one of the most puzzling features connected with these experimental arterial lesions, namely, "plasma alteration," but do not explain how "plasma alteration" augments the process.

5. The original conjecture "that the lesions may be due to a hypersensitivity to some constituent of the blood of one or more of the donor dogs"¹ is no longer tenable. The problem apparently becomes one of disturbed metabolism and resolves itself into two factors: (1) a dietary factor and (2) a factor associated with renal insufficiency.

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DESCRIPTION OF PLATES

PLATE 21

Necrotizing arterial lesions in dogs. All sections were taken from dog no. 40-84 and were stained with hematoxylin and eosin.

FIG. 1. Acute necrotizing arteritis at point of origin of innominate artery from aorta. $\times 100$.

FIG. 2. Acute necrotizing left auriculitis. $\times 100$.

FIG. 3. Acute necrotizing arteritis of pulmonary artery. $\times 100$.



Holman

Experimental Necrotizing Arteritis. IV

PLATE 22

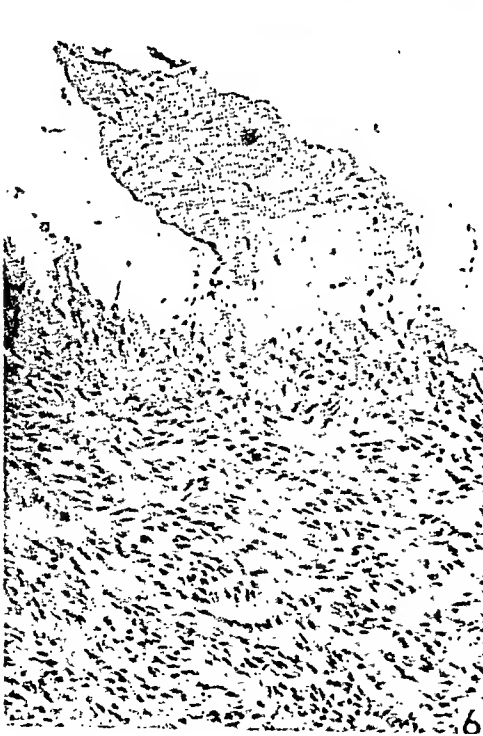
Necrotizing arterial lesions in dogs. All sections, except that used for Figure 5, were stained with hematoxylin and eosin.

FIG. 4. Dog no. 40-79. Acute necrotizing arteritis of common carotid artery. $\times 128$.

FIG. 5. Dog no. 40-79. Another section of common carotid artery, stained with Verhoeff's elastic tissue stain. The "normal" undulating pattern of the internal elastic membrane and medial elastic tissue is replaced with "straight lines."
 $\times 128$.

FIG. 6. Dog no. 40-68. Healed lesion in intima of pulmonary artery. $\times 128$.

FIG. 7. Dog no. 40-68. Healed lesion in artery in lung. $\times 150$.



STUDIES ON EXPERIMENTAL RICKETS IN RATS

IV. THE RELATION OF RICKETS TO GROWTH, WITH SPECIAL REFERENCE TO THE BONES *

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In former papers (1934, 1938 and 1939) we have described the changes in the bones of rachitic rats, with special reference to the epiphyseal cartilage, both in the active phase of rickets and during healing of the disease. Inasmuch as the osseous changes in these rats have been studied and described in considerable detail, it seems desirable to present our observations upon the growth pattern of these same rats, as it is modified during the development and healing of rickets, with special emphasis upon the growth of the bones. It is well known that experimental rachitogenic diets inhibit growth to varying extents. The observations recorded here give further information concerning growth as affected by the Steenbock-Black diet '2965, so widely used in experimental work. A significant feature of the present studies lies in the fact that we have traced the progress of rickets and the growth of the bones in individual rats by the use of frequent roentgenograms of the living rats.

MATERIAL AND METHODS

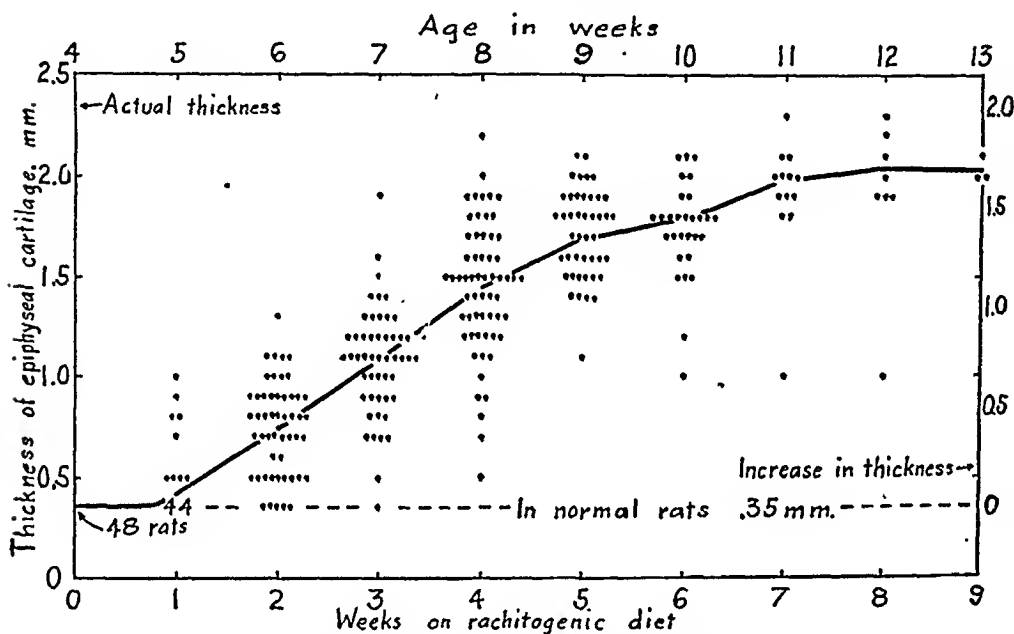
Rickets was produced by the Steenbock-Black rachitogenic diet 2965. Healing was produced in most of the rats by irradiated ergosterol (Viosterol), though in a smaller number cod-liver oil was used. The rachitogenic diet was begun at the age of 4 weeks; the curative treatment at 8 or 9 weeks of age, after rickets had become well established.

Growth records were made of 135 rats sacrificed for microscopic study at ages of 4 to 13 weeks, including 22 rats on a balanced ration and 113 on the rachitogenic diet. The records included 678 roentgenograms of the 135 rats. Of 58 rats roentgenograms were taken weekly or more often; from the remaining 77, at less frequent intervals or only at the time of sacrifice. The microscopic studies covered the bones from 112 of these rats. Line test studies were made of 37.

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The roentgenograms were taken of entire rats as they lay, ventral side down, relaxed under light ether anesthesia. Measurements made on x-ray films under magnifications of a few diameters were found to be fairly accurate and served to show general trends of growth very well, though there were some minor irregularities due, presumably, to inaccuracies in measurement, or to varying attitudes of the bones. Certain corrections were made to compensate for the angles between the several bones and the photographic film. The length of the ver-



Text-Figure 1. Scattergram showing rate of development of rickets in rats as measured by the increase in thickness of the epiphyseal cartilage in the head of the tibia, based upon 90 rats on the Steenbock-Black diet 2965 (359 separate x-ray records). The individual points represent weekly records. The curve shows the average value for each week.

tebral column is the least reliable of the measurements, because the vertebral column is subject to varied degrees of arching for which there is no obvious correction.

We have recorded graphically the growth of each rat, using appropriate arbitrary scales which make the several items readily comparable and at the same time show the actual values as measured. Text-Figures 2-4 show 3 rats with different habits of growth.

THE RATE OF DEVELOPMENT OF RICKETS

As an index of the severity of rickets we employed the pathologic thickening of the epiphyseal cartilage in the head of the tibia, a widely used and easily measured anatomic feature of the disease. The weekly values for the thickness of this cartilage were plotted for 90 rats, using 359 roentgenograms, 261 of which showed some thickening of the cartilage (Text-Fig. 1). This graph shows the average rapidity

of development of rickets and the extremes for each week. In only 9 of 53 rats was there any measurable thickening after 1 week on the rachitogenic diet, whereas all but 5 showed at least slight thickening after 2 weeks. The curve shows that the thickening progressed rather rapidly in the earlier weeks, but more slowly in the later weeks, until a maximum of about 2 mm. was attained. Records for each individual rat showed the same general trend, although there were differences in the exact form of the curves.

This graph (Text-Fig. 1) does not definitely answer the question whether the cartilage wholly ceased to thicken, or merely grew very slowly during the later weeks. The individual records, however, indicated that in some rats the cartilage definitely continued to increase in thickness for 8 or 9 weeks on the diet, while in others there was no measurable increase after about the fifth week. Nor has it been clearly demonstrated that mitosis of the cartilage cells wholly ceased in some rats with advanced rickets, although our microscopic studies showed that it at least became very slow. The uncalcified condition of the thickened cartilage clearly allows some compression (emphasized by Park, 1939), which would prevent actual thickening even though there might be a slow multiplication of cells.

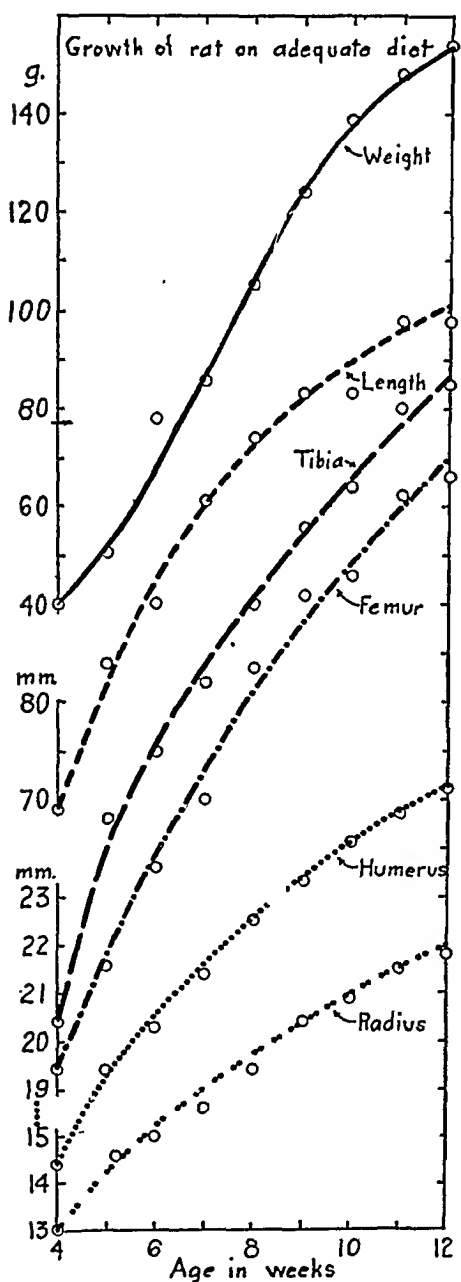
The few scattered records well below the main group in Text-Figure 1 represent a few rats with very mild rickets. Microscopic study showed that in these rats, calcification did not wholly cease, nor was cartilage removal entirely suspended. Cartilage removal did, however, lag considerably behind growth, so that the cartilage became somewhat, but not greatly, thickened. The thinner cartilage in such cases of mild rickets does not indicate less rapid cartilage growth than in severe rickets but, on the contrary, more rapid growth as shown by the observation that the elongation of such bones was definitely greater than in rats with severe rickets. Unfortunately, no weekly roentgenograms of these rats were taken, but only the terminal picture, as seen at the time of their sacrifice.

RICKETS IN RELATION TO GROWTH

The Weight of Rachitic Rats

The rats on the rachitogenic diet gained weight less rapidly than did the controls, an observation in accord with general experience with this and other commonly used rachitogenic diets (Text-Figs. 2, 3 and 5). In general it may be said that the more severe the rickets, the more the growth curve, after 5 or 6 weeks, fell below the normal. But the rats in which rickets developed most rapidly for the first 3 or 4 weeks were neither the heaviest nor yet the lightest, but rather the

intermediates. This relation is shown when the severity of rickets (thickening of the epiphyseal cartilage) is plotted against the gain in weight for rats of a given age (*e.g.*, at 7 weeks, as in Text-Fig. 6). This scattergram shows for the main group of rats an evident, positive correlation; that is, the rats which showed the greatest gains in weight since the beginning of the rachitogenic regimen had developed the most advanced rickets. But there was a minority, including rats much heavier than the main group, in which rickets was less advanced.

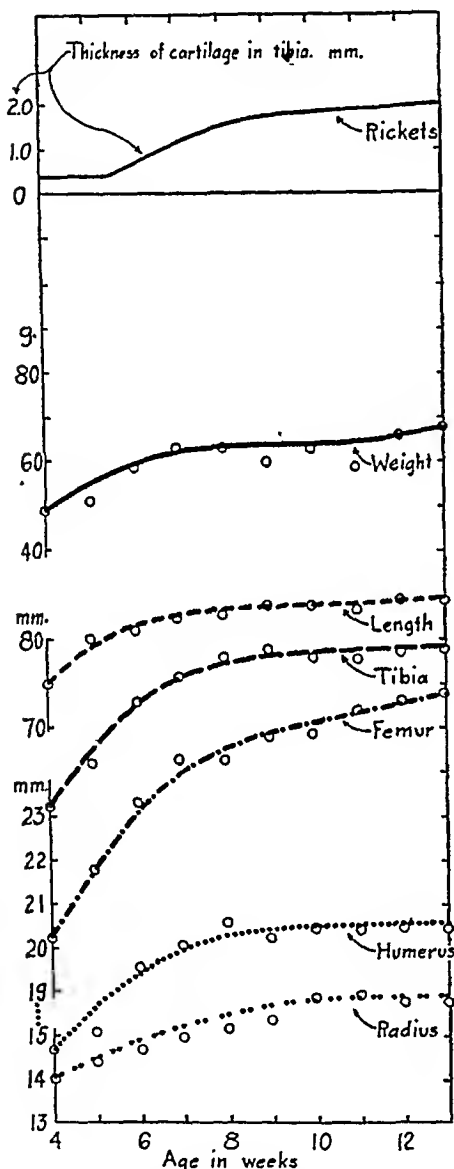


Text-Figures 2-4. Graphs of 3 rats showing typical modes of growth for weight, length of body and length of leg bones. Weekly records are shown by circles; growth trends by smooth curves. At the top of Text-Figures 3 and 4 are shown the development of rickets (as measured by the thickness of the epiphyseal cartilage) and the progress of healing (as measured by Bourdillon's 12-stage scale). All of these values are plotted to approximate arbitrary scales which make the separate items readily comparable.

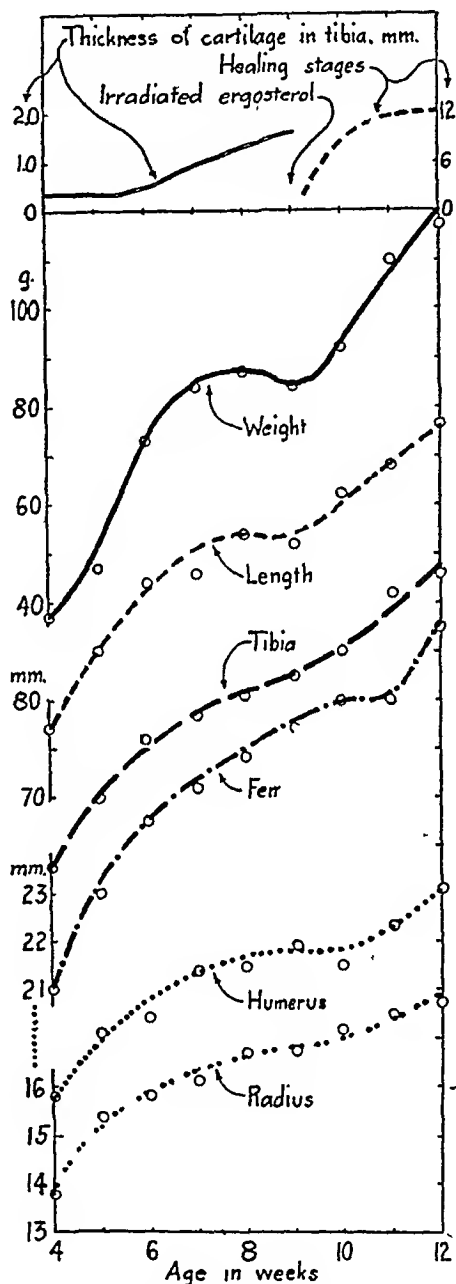
Text-Figure 2. Rat no. 130, male, maintained on a balanced diet. The growth is well within the limits for normal, healthy rats.

This peculiar relation was shown equally well in these rats after 2, 3, or 4 weeks on the diet.

But when for these same rats the growth of the leg bones (*e.g.*, the



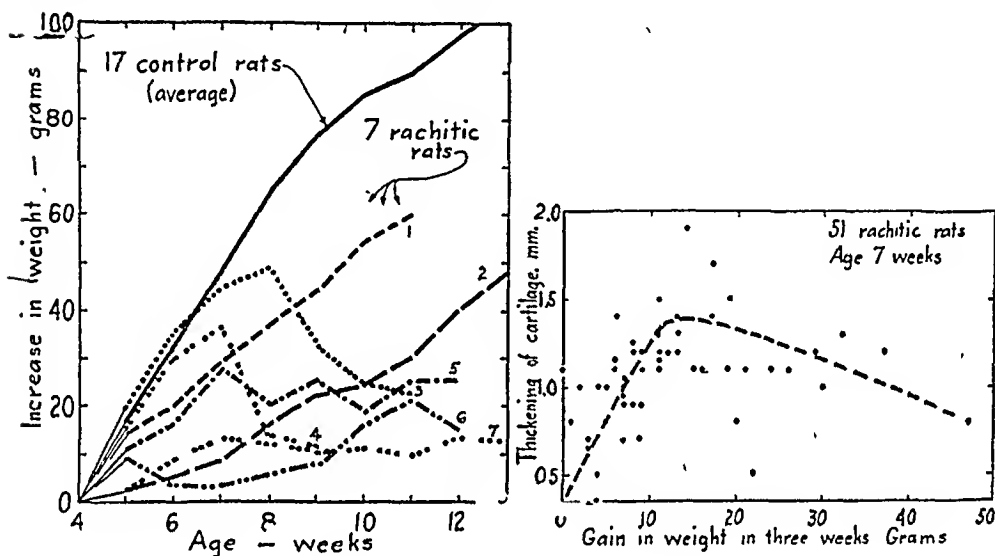
Text-Figure 3. Rat no. 155, male, maintained on diet 2965. Persistent, severe rickets. Growth was somewhat subnormal even during the first week on the experimental diet, and almost ceased after 5 weeks.



Text-Figure 4. Rat no. 134, female, maintained on diet 2965 throughout, but given irradiated ergosterol after 5 weeks. After about 5 weeks on the rachitogenic diet the growth rate became much reduced, but it was accelerated when healing was produced by the administration of vitamin D.

tibia) was plotted against the rate of development of rickets, all the records fell in a compact group showing a direct correlation. The heavy rats shown in Text-Figure 6 were evidently those which were able, to a high degree, to resist the development of rickets and to escape the degree of emaciation which accompanied rickets in the others.

The increase in weight of rachitic rats did not follow any definite pattern, but assumed a wide diversity of forms (Text-Fig. 5). The growth patterns were not distributed at random, but all rachitic mem-



Text-Figure 5. Graphs showing average increase in weight of 17 normal rats and of 7 individual rachitic rats (no curative agent fed and no spontaneous healing). These rats illustrate the variety of growth patterns assumed by rachitic rats. Each one is typical of a considerable number of rats of both sexes.

Text-Figure 6. Scattergram of 51 rats (age 7 weeks) showing relation between gain in weight and severity of rickets after 3 weeks on diet 2965. About 40 of the rats fall in a group showing positive correlation between gain in weight and severity of rickets (thickness of epiphyseal cartilage). The remaining heavy rats suggest a reverse correlation.

bers of a litter usually grew similarly, as indeed did those in all litters at about the same time. The growth pattern changed from time to time, but there was not observed any pattern definitely characteristic of any season of the year. In general, in accord with common experience, rickets was more severe and the growth less rapid in winter than in summer, but this was by no means a clear-cut rule. Zucker, Hall and Young (1941), in a recent paper, emphasized these "seasonal or recurrent, aperiodic variations" which must be considered in making any kind of comparative growth study upon rachitic rats. Such variations have made it necessary to use great caution in drawing conclusions from our averages.

The reason for such variations in growth response to the rachitogenic diet is not evident. Temperature may be a minor factor, but rats kept at abnormally high temperature did not yield any definite information concerning the effect of temperature. In respect to the severity of rickets in relation to temperature, Tourtellotté and Bacon (1935) are not in agreement with the conclusions of Guerrant, Dutcher and Crowthers (1937), which would suggest that there cannot be any considerable influence of temperature. Nor were the differences in growth patterns distributed according to sex; the females grew less rapidly than the males, but the various patterns were distributed between the two sexes. Nor did the growth pattern bear any detectable relation to the weight of the young rats at the beginning of the experimental period, nor to the previous diet of the mothers. Presumably some sort of undetected differences in the rachitogenic diet must be responsible.

The Growth of the Leg Bones

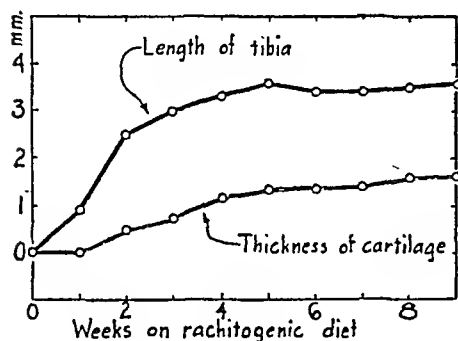
The growth of the leg bones was retarded greatly in rachitic rats. In a given rat all the leg bones followed the same general pattern and all were retarded to about the same extent (Text-Fig. 3). The bones of different rats did not show the same diversity of growth patterns as did the weights of the same rats, possibly largely because the length of bones cannot be subject to the same fluctuations as the weight of the animal. The only conspicuously different growth patterns of bones were those characteristic of severe rickets, mild rickets and healing rickets (Text-Figs. 8, 9 and 10).

The weekly measurements showed that the bones of rachitic rats suffered progressive retardation until, after about 4 weeks on the diet, the growth became very slow, and in some rats ceased entirely. It is clear, however, that there was no definite and universal growth limit at the age of 7 weeks (3 weeks on the diet) as there seemed to be from our earlier estimations which were not checked by weekly roentgenograms (1934), nor at about 11 days as recorded by Park (1939).

The Tibia and Its Epiphyseal Cartilage

The growth of the tibia and its epiphyseal cartilage gives a clear illustration of the way in which rickets influences the elongation of bones through its effect on the epiphyseal cartilage. In rats over 4 weeks of age only the cartilage in the proximal end of the bone need be considered, because by that time the cartilage in the distal end has ceased to be a factor in the growth of the bone.

When the elongation of an average rachitic tibia and the thickening of its epiphyseal cartilage were plotted to the same scale and from a common origin, the two curves diverged for a time, but soon became nearly parallel, and throughout the greater part of their courses were separated by about 2 mm. (Text-Fig. 7). During the first week the tibia showed little effect of rickets and continued to grow in length



Text-Figure 7. Rat no. 155, male, with persistent severe rickets. The curves for the growth of the tibia and for the increasing thickness of the epiphyseal cartilage are plotted from a common origin. The two curves are divergent until cartilage removal ceases (for about 2 weeks), after which they become nearly parallel, being separated by about 2 mm. The parallel relation persists as long as severe rickets continues.

about as in normal bones. During these days the cartilage became no thicker, because its growth was normally utilized in the production of bone in the end of the growing shaft. But when the advance of calcification into the cartilage ceased and cartilage removal was suspended, the calcified shaft of the bone also ceased to grow longer. The uncalcified cartilage, however, continued to grow and, because none of it was being removed, increased in thickness. Thus it comes about that the elongation of severely rachitic bones is expressed wholly in the increased thickness of the cartilage rather than in the normal elongation of the osseous shaft. The parallel course of the two curves in Text-Figure 7 shows this relation.

The growth relations shown in Text-Figure 7 represent about the average condition in severe rickets. The departures from this average condition involve a time factor (varying time elapsing before calcification and cartilage removal begin to lag behind growth, and before these two processes wholly cease); and a growth factor (varying rates of growth). The time factor determines how soon the curves become parallel; the growth factor, the distance between their parallel portions.

On the average, the tibia had grown in length 1.5 mm. (extremes of 0.5 mm. and 2.7 mm.) before the cartilage began to thicken; and had grown 2.0 mm. (extremes of 1.5 mm. and 3.0 mm.) before calcification and cartilage removal wholly ceased. The continued thickening of the cartilage after the cessation of its removal added an average of about 1.7 mm. to the length of the bone, a value from which the variations in either direction were not great. Thus, in rats with severe and persistent rickets, the total average growth of the

tibia during the rachitogenic regimen was 3.7 mm. (2.0 mm. plus 1.7 mm.), with extremes of 3.2 mm. and 4.7 mm. This is about one-third of the normal growth, inasmuch as during the same weeks (up to the age of 12 weeks) the average growth of the tibia in the control rats was about 10 mm. (Text-Fig. 10). The behavior of the tibia as described above may be considered as characteristic of the long bones in general.

The Growth of the Vertebral Column

The growth of the body in length (the growth of the vertebral column as measured from the base of the skull to the caudal end of the innominate bone) showed deficiency, as did the leg bones (Text-Figs. 2, 3 and 9), though the varied amount of arching, especially pronounced in rats with very severe rickets, made roentgenograms unreliable in the detection of small increments of growth. In the control rats the vertebral column continued to grow steadily, until at the age of 12 weeks it had made an average growth of 47 mm. over its length at 4 weeks. In the rachitic rats the growth of the vertebral column showed progressive retardation, as did the leg bones. After about the ninth week its growth was very slight, and by the end of the twelfth week it showed an average increase of only 12 mm. since the fourth week (about one-fourth the normal growth).

During the experimental period (4 to 12 or 13 weeks of age), the leg bones of the control rats grew relatively less rapidly than did the vertebral column, so that the legs became proportionally shorter. In our rachitic rats the relative retardation of the vertebral column was greater than that of the leg bones, to the extent that in them the legs and the vertebral column accomplished about the same relative growth.

It is not surprising that rickets should affect the growth of the legs and the vertebral column to different extents, because the growth of each leg is the result of rapid growth of very few cartilages (two, exclusive of those in the foot), whereas the growth of the vertebral column is the summation of the growth of sixty cartilages, each of which grows very slowly. It is conceivable that rickets might affect the two types of cartilage to different extents, but it is not evident why the retardation should be greater in the cartilages which normally grow slowly than it is in those which grow rapidly. In this connection it is of interest to note that in two series of rats on two different non-rachitogenic diets (Outhouse and Mendel, 1933) the leg bones in the more slowly growing series were longer with respect to the body than in those which grew more rapidly; a result similar to that observed in our rachitic rats.

THE HEALING OF RICKETS IN RELATION TO GROWTH

Measuring the Healing Process

The degree of healing of rickets, as commonly measured by the line test, is an indication of the extent of resumed calcification. This calcification is intimately associated with the extensive tissue reorganization of the healing process (Dodds and Cameron, 1938). Roentgenograms, although not as extensively used as the line test, afford another very useful means of observing the extent of calcification, and have the advantage of applicability to the living rat. Some writers have advocated their wider use (Poulsson and Lövenskiöld, 1928; Bourdillon, Bruce, Fischmann and Webster, 1931), and have described useful methods. Our estimation of healing is based upon the 12-stage scale of Bourdillon and co-workers (Dodds and Cameron, 1938).

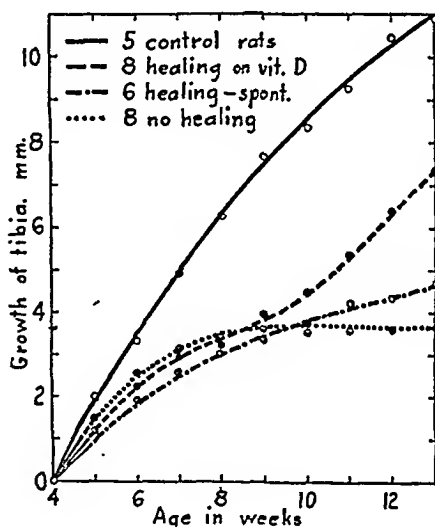
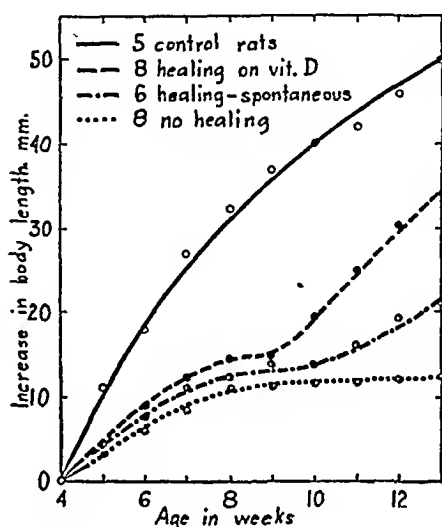
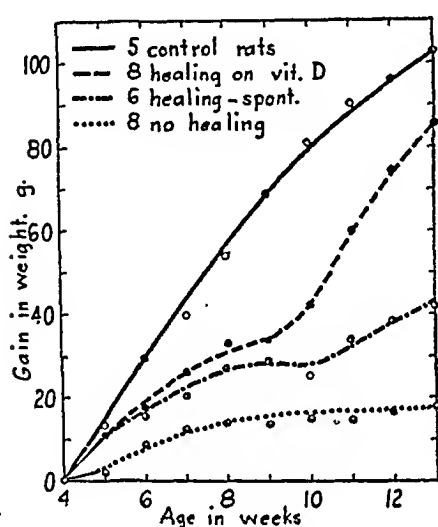
Graphs from roentgenograms of the head of the tibia showed that healing began promptly after the administration of vitamin D, and that it was often practically complete in 2 weeks, although a longer time frequently was needed. Text-Figure 4 includes graphs showing the progress of healing in a typical rat under the influence of vitamin D. The roentgenograms also revealed cases of spontaneous healing which began without the use of vitamin D. No such case of spontaneous healing came to completion in any observed rat, but after a week or two gave place to renewed incidence of rickets. We interpret these cases of spontaneous healing as representative of an intermediate condition between severe rickets and the mild form in which cartilage removal does not wholly cease.

Healing and Accelerated Growth

When healing took place under the influence of vitamin D there was a marked improvement in growth, both in the weight of the rats and in the length of the bones. Comparison of Text-Figures 3 and 4 shows resumed growth in a typical rat. A comparable acceleration was seen in every rat which was allowed to live long enough to bring healing to practical completion.

We have recognized and plotted four general types of growth, the types corresponding to the severity of rickets (Text-Figs. 8-10). These types are distinguishable with equal clearness with respect to increase in weight, growth of the leg bones and growth of the vertebral column. These types are as follows: (1) Normal growth seen in rats on a balanced ration. (2) Serious and persistent growth retardation seen in those rats on the rachitogenic diet which developed severe and persistent rickets. (3) Marked retardation followed by acceleration

in the later experimental weeks, seen in rats which were undergoing healing under the influence of vitamin D. Not all rats undergoing healing on vitamin D showed as great growth acceleration as the eight included in the average shown in these figures, but it is true that every such rat showed more rapid growth than those in the same litters which did not receive the vitamin D. (4) An intermediate group vacillated between severe rickets and spontaneous partial heal-



Text-Figures 8, 9 and 10. Average growth curves for normal rats and for rats showing three different degrees of rachitic severity (compare Text-Figs. 2-4). Normal rats grew steadily and rapidly. Rats with persistent rickets grew with increasing slowness until growth almost ceased. Rats with spontaneous healing grew slowly but showed a tendency to acceleration in the latter weeks. Rats under the influence of vitamin D showed marked acceleration after healing took place. Compare Text-Figure 8, increase in weight; Text-Figure 9, growth of vertebral column; and Text-Figure 10, growth of tibia.

ing, with recurrent rickets. The growth trend was also intermediate between those of the two preceding groups of rachitic rats.

To these four might be added a fifth group, including a few rats with very mild rickets (shown in Text-Fig. 1), in which calcification was less seriously deficient and removal of the epiphyseal cartilage was never wholly interrupted. The growth of these rats was only moderately retarded (Text-Fig. 5, curve 1). There were no weekly roentgenograms of these rats, but the final picture shows that the bones accomplished distinctly greater growth than in the other rachitic groups.

DISCUSSION

Inasmuch as growth is much retarded when experimental rickets develops in rats and is resumed when healing takes place, the conclusion might be drawn that reduced growth is part of the rachitic condition. On the other hand, when vitamin D is added to the rachitogenic diet 2965 throughout the experimental weeks, though no anatomic evidences of rickets develop, yet the growth is no better than in rats receiving diet 2965 alone. This result has been observed in our rat colony and has been pointed out also by Kunde and Williams (1927) and by Zucker, Hall and Young (1941) and others. Such results might suggest that the growth deficiency is due directly to diet deficiency rather than to rickets. But the answer is not as simple as that.

It has been observed by various workers (among them Osborne and Mendel, 1918) that inadequacy of phosphorus is a common cause of reduced growth; and more recently it has been pointed out that phosphorus deficiency is the chief growth-limiting factor of the rachitogenic diet 2965 and other high-calcium, low-phosphorus diets (Shohl and his co-workers, 1928, 1933 and 1939, and other workers). When renewed calcification takes place in the healing of rickets under the influence of vitamin D, it is presumably the better utilization of the scant phosphorus in the ration that makes possible the more rapid growth of all bodily tissues. But it is not evident why the rachitogenic diet with vitamin D added, though preventing rickets, does not produce better growth than the rachitogenic diet alone. This is especially puzzling in consideration of the marked growth acceleration which accompanies healing under the influence of vitamin D.

Day and McCollum (1939) and Follis, Day and McCollum (1940) found that with a purified rachitogenic diet, very low in phosphorus, the very grave growth deficiency was due, in part at least, to inanition, because the rats did not eat enough of the ration. We found that in

our colony the rats on the rachitogenic diet did consume a little less food than those on the stock diet, but we observed no evidences of inanition. Moreover, the rachitic rats actually consumed more food per unit of body weight than did the controls. The quality of the food rather than the quantity was responsible for the deficient growth.

Inasmuch as phosphorus deficiency is known to produce both rickets and subnormal growth, it would seem that the growth deficiency should be considered a part of low-phosphorus rickets. It is generally agreed that vitamin D produces an increased net absorption of phosphorus from the alimentary tract of rachitic rats which is sufficient to relieve the rachitic condition and to bring about somewhat better growth. But as long as the phosphorus in the ration is markedly low, the improvement in rachitic rats does not result in fully normal growth nor, according to Zucker, Hall and Young (1941), in a completely normal mineral content of the bones.

Vitamin D is not of itself a growth-promoting agent; it merely acts to increase the available phosphorus. Of interest in this connection are the results of Stearns, Jeans and Vandecar (1936) and Slyker, Hamil, Poole, Cooley and Macy (1937), who observed in over 500 infants an increase in growth rate upon the addition of vitamin D to a ration already adequate enough to prevent rickets. Presumably the vitamin D was effective in increasing growth through its power to bring about greater economy of the phosphorus in the ration.

The growth modifications observed microscopically in the rachitic epiphyseal cartilage (decreased cell multiplication and reduced growth of the cells in the columns) would indicate that in rickets there must be a slowing of growth of the bones, quite apart from actual gross measurements of this deficiency (Dodds and Cameron, 1934). Since the observed deficiency in growth takes place in the zones of the cartilage which even in normal bones are not calcified, one is led to wonder if the subnormal weight of the animals may not be due to a similar effect upon other noncalcified tissues. This growth-limiting effect upon the epiphyseal cartilages of bones is quite distinct from the commonly recognized phase of rickets (inadequate calcification of bone and cartilage) and constitutes a separate phase of the disease, presumably also brought about directly by the phosphorus scarcity. This limitation of cartilage growth has not been recognized by most students of rickets, and the excessive accumulation of uncalcified cartilage has led some to the erroneous conclusion that there is an actual acceleration in cartilage growth.

Investigators in the field of experimental rickets have generally recognized that in rachitogenic diets the scarcity of phosphorus is

not the only growth-limiting deficiency, though the others have not been so definitely defined. For this reason the growth deficiency of rats receiving the diet 2965 probably is greater than might be expected in uncomplicated low-phosphorus rickets. The existence of these other deficiencies in this diet has recently been clearly demonstrated by Zucker, Hall and Young (1941), who found that the addition of adequate amounts of phosphate to this ration still left it quite inadequate to produce normal growth. In an attempt to find a better rachitogenic diet, these workers have devised a high-calcium, low-phosphorus diet, no. 803, which produces as severe rickets as diet 2965 with less retardation of growth. Moreover, when phosphate in proper amount is added to diet 803, it becomes practically a complete diet and produces very nearly normal growth in rats. But like diet 2965, this diet does not show improved growth production upon the addition of vitamin D alone.

Though it is generally recognized that growth deficiency accompanies experimental rickets in rats, there has been no general agreement as to the impairment of growth in rachitic human infants. Hess (1929) stated that in the common moderate case of human rickets there is usually no decrease in the growth rate, though in the extreme form of rickets impairment of growth may be encountered. He quoted Wimberger (1923), who studied the growth of the tibia and other long bones by roentgenograms, and came to the conclusion that growth does not suffer any impairment through rickets.

On the other hand, we find statements like that by Mueller (1924), who said it is well known that in high-grade rickets, growth in length suffers to a conspicuous degree. Feer (1922) stated that rachitic children are always shorter than others, even if there is no evident distortion of the bones. Holt and Howland (1929) said: "A change which has not been sufficiently emphasized is arrested growth of the long bones; this is the most characteristic feature of rickets." Reed, Struck and Steck (1939) emphasized the importance of even very mild rickets because of the depressing effect upon growth. Indeed, the studies of Slyker and co-workers and of Stearns, Jean and Vandecar seem to indicate that infants suffer growth deficiency when there is an inadequacy of phosphorus which is not quite severe enough to produce rickets.

On the whole, it would seem that we must accept the view that rachitic infants commonly suffer growth deficiency. It is probable, however, that the deficiency is by no means as great as that observed in rachitic rats, otherwise there would scarcely be any question about its occurrence. No doubt the division of opinion may also be due in

part to the impossibility of carrying out extensive experimental studies under rigidly controlled conditions.

The seemingly different magnitude of growth response between rachitic infants and rats may possibly be explained in part by the different dietary requirements necessary for the production of rickets in the two species. Rats do not develop rickets unless, in addition to deficiency in vitamin D, there is also a distinctly unfavorable ratio of calcium and phosphorus in the diet, usually high-calcium and low-phosphorus (Hess, 1929; Shohl, Bennett and Weed, 1928; and others). Infants, on the other hand, commonly develop rickets on diets deficient only in vitamin D, or when deprived of solar radiations, even though calcium and phosphorus in the diet may be adequate. Zucker, Hall and Young (1941) expressed the situation by saying that in rats rickets is primarily a phosphorus deficiency (though it can be largely corrected by vitamin D), while in infants it is purely an inadequacy of vitamin D, usually in the presence of adequate calcium and phosphorus in the diet. Thus, infants often have rickets on a diet of cow's milk, whereas rats will not become rachitic when there is cow's milk in their diet. On such a diet infants may suffer physiologic scarcity of phosphorus, even though satisfactory amounts of phosphorus are actually presented in the food. The phosphorus scarcity so produced is seemingly usually not severe enough to retard growth as seriously as when rickets is produced experimentally in rats.

SUMMARY

These studies are based upon 135 albino rats, in most of which rickets was produced by the Steenbock-Black diet 2965. The special feature of the study is the tracing of the development and healing of rickets and the growth of the bones by weekly roentgenograms. Graphic methods are used to show rickets, healing and growth.

The rachitic rats were very subnormal in weight, but their growth did not follow any single pattern.

The growth of the leg bones and of the vertebral columns of the rachitic rats was greatly retarded. The retardation of the vertebral columns was relatively greater than of the leg bones.

The epiphyseal cartilage of the tibia (typical for all long bones), during the first week or two on the rachitogenic diet continued to make its contribution to the length of the shaft of the bone, but in decreasing amount. After about the third week the shaft ceased to elongate, and the pathologic thickening of the epiphyseal cartilage and the elongation of the bone became equal and identical.

When rickets healed under the influence of vitamin D, both increase in weight and growth of bones were accelerated to a marked degree. When spontaneous healing took place there was a less marked acceleration.

We believe that the reduction in growth rate is a part of low-phosphorus rickets, and, like other phases of rickets, is produced by phosphorus deficiency. Other growth-limiting factors in most rachitogenic diets are also recognized, but these are not concerned in the production of rickets.

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A FATAL DISEASE OF MIDDLE-AGED MICE CHARACTERIZED BY MYOCARDITIS ASSOCIATED WITH HEMORRHAGE IN THE PLEURAL CAVITY *

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In routine autopsies of a large colony of mice maintained in this department for the study of leukemia, 107 mice were found to have died during a period of approximately 3 years with a well characterized disease complex still present in our colony which, to our knowledge, has not previously been described. Although we have been unable to determine the etiology of this disease, it seems desirable to describe it because it apparently constitutes a well defined entity that is of sufficient interest to warrant further investigation.

INCIDENCE; AGE AND SEX RELATIONSHIP

The colony is comprised of from 5000 to 7000 mice per annum, with a mortality of about 3000 per annum. The disease occurred throughout the year, affecting adult male mice. At the early phase of this study 5 female mice were recorded as having died of this disease, but the hearts of these animals were not sectioned, and since this disease has been studied carefully it has not been found among female mice. The age distribution was as follows:

Age in months	Less than 5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	22-23	Over 23
Number of mice	4	4	8	16	16	19	12	13	10	2	1

This tabulation shows that the greatest mortality occurred between the 10th and 15th month, with a maximum at 14 months. The greater part of our colony is under 12 months of age. All of the last 14 cases observed occurred among males, and 13 were of the Rf stock. Their age varied from 8 to 19 months, with an average of 13 months.

ANATOMIC CHANGES

Gross examination indicated that exsanguinating hemorrhage into the pleural cavity was the immediate cause of death in most instances. Coexisting myocardial and epicardial hemorrhage was common, while the lungs were, as a rule, free from hemorrhage; the vascular rupture

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usually occurred near the origin of the great vessels. Peritoneal hemorrhage was noted in only 1 animal. Testicular hemorrhage, however, occurred frequently and was recorded in 27 mice. This figure, however, is inaccurate. More recently all testes have been sectioned and hemorrhage, although minute, is found in almost every case. Testicular hemorrhage unaccompanied by pleural hemorrhage was recorded at first in 11 cases, but in none of these was the heart examined microscopically; myocarditis was doubtless overlooked in several instances. More recently, the hearts have been examined microscopically in every case, and advanced myocarditis found, which may have accounted for the death of the animals. Thrombi distending the auricles of the unopened heart were noted in several cases.

The only other conspicuous change on gross examination was ischemia of the liver with yellowish discoloration, due to exsanguinating pleuro-pericardial hemorrhage, and moderate to advanced fatty degeneration. In many cases, the liver appeared smaller than normal. In general, the animals appeared to be in an excellent state of nutrition; many appeared obese and all were well developed.

Heart. Microscopic examination showed acute and subacute interstitial myocarditis in 23 of 25 mice that were examined. Since the bodies from the 2 mice without myocarditis have been discarded, it is impossible to state whether or not pleural hemorrhage in these cases was due to a different disease from that described here. The most frequent histologic picture was a diffuse infiltration by polymorphonuclear and mononuclear leukocytes of the muscle of auricles and ventricles (Figs. 1, 2 and 4), most extensive in the region of the auriculoventricular junction. The cardiac fibers were often widely separated by edema fluid. In areas where the inflammation was most intense, there was occasionally considerable necrosis of the heart muscle. In several animals, the process was extensive and a site of rupture was demonstrated in the auricular wall. The inflammatory process always involved the myocardium; the pericardium about the base of the heart was also frequently involved (Fig. 3), as was the endocardium, but to a lesser extent. The serosal and endocardial layers were elevated in several places by both inflammatory exudate and hemorrhage. Antemortem thrombi were occasionally attached to the auricular endocardium. The valve leaflets, as seen in sections occasionally, showed slight or moderate inflammation and hemorrhage. Frequently the myocardial changes were more chronic and characterized by considerable numbers of mononuclear leukocytes, lymphocytes and fibroblasts (Fig. 4). Occasionally, sheetlike masses of large mononuclear cells were seen, with "owl-eyed" nuclei, as in Aschoff

bodies. The cells were evidently Anitschkow "myocytes"¹ or "myocardial reticulocytes."² Characteristic Aschoff bodies were not seen. Fibrosis unassociated with inflammation was never observed.

Serial sections of the heart and lungs were made from 3 animals and the site of rupture was demonstrated in each. It was situated in either the auricle or in an adjacent orifice of a large vein. No parasite or micro-organism could be demonstrated with certainty.

Lungs. The most constant and conspicuous lesion was a hyaline degeneration of the smooth muscle in the walls of the medium-sized pulmonary arteries. It was observed in 11 of 27 mice. This alteration in the vessel was not associated with hemorrhage. A moderate degree of hyaline degeneration of arteries was observed in the lungs of only 3 of 26 control mice of the same age and sex. Chronic bronchitis and bronchiectasis were observed frequently, but the incidence was not greater than in the controls. Rarely, numerous erythrocytes were seen in the alveoli.

Testes. Extensive hemorrhage between the seminiferous tubules was a characteristic occurrence. Grossly, as a rule, only one testis seemed involved, but on microscopic examination the hemorrhage was usually bilateral (Fig. 5). There was frequently a slight degeneration of the tubules, but spermatogenesis was not prevented. Often small or moderate numbers of polymorphonuclear leukocytes were present; this was not a constant feature, and a few polymorphonuclears were also found in sections of testes with no hemorrhage. No rupture of any blood vessel was demonstrated with certainty although in 4 instances moderate degeneration was observed in the walls of the blood vessels. In several cases there was calcification of the media of large arteries in both capsule and parenchyma of the testes (Fig. 6). There seems to be no relation between this medial calcification and the acute fatal illness. While the cardiac lesions often indicated a subacute process, those of the testes were always acute.

Other Organs. The kidneys, spleen, adrenals, bone marrow, hypophysis and brain from a representative group of mice dead with this disease complex were examined. No constant pathologic change was noted in these organs.

EPIDEMIOLOGIC OBSERVATIONS

The mouse colony occupies four large rooms on two floors. Four different inbred stocks of mice and hybrids were studied. The disease occurred in all stocks and hybrid mice, and its incidence was roughly proportional to the number in each stock. Thirty mice with this disease were of the Rf stock and only 3 of stock Ak, but most mice of the

latter stock die within approximately 10 months, mainly of leukemia, whereas the peak of the mortality curve among Rf mice is at 15 months. Thus, many more mice of the Rf stock reached the period of susceptibility to the disease studied. Similarly, most hybrid mice are long-lived and most examples (63) of this disease were found among hybrids.

To obtain information on the possible spread of the disease in the animal colony, the cages in which mice had died with this disease were marked without changing their location and without sterilization. There was no evidence to indicate spread of the disease within the cage or from cage to cage, although several cases occurred in the same cage at widely varying intervals.

BACTERIOLOGIC EXAMINATION

The hearts from 8 cases of myocarditis were ground and cultured on the usual laboratory media. Inoculations were also made on 30 per cent horse serum agar for the detection of pleuropneumonia-like organisms; however, none were isolated. The organisms grown from a few cases were usually *Staphylococcus albus* and Gram-negative bacilli. These were regarded as postmortem invaders.

A hemorrhagic testis from 1 mouse with extensive myocarditis was ground in broth, passed through a Mandler regular filter and inoculated onto the chorioallantoic membrane, but no lesion was observed.

TRANSMISSION EXPERIMENTS

The inoculations of heart tissue from 7 mice with myocarditis into a total of 74 healthy mice by the intravenous, intracerebral, or intraocular routes were without results. The myocardial tissue from the 8th mouse was injected into 4 normal mice by the intrapulmonary route, and into 5 by the intravenous route. Of the mice injected intravenously, 1 died within 24 hours and the heart was negative; another died within 10 days with both gross and microscopic evidence of myocarditis. Cultures of the heart muscle yielded no growth. The tissue was passed to 5 more mice. All died, however, as a result of *Bacillus typhi murium* infection within the incubation period of myocarditis. Three killed after 24 days had no evidence of disease. Of those inoculated by intranasal instillation, 1 died on the 18th and 1 on the 24th day, and although there was no gross evidence of myocarditis, it was present on histologic examination. The remaining 2 were killed on the 24th day and there was no evidence of disease. The inoculated animals that showed myocarditis had neither pleural nor testicular hemorrhage, and on subsequent passages the mice of

this series died from infection with *B. typhi murium*. It is probable that this myocarditis was attributable to *B. typhi murium* that was present in a small number of mice purchased for transmission experiments. This infection had not been encountered in our colony and the inoculated animals were kept in a different room.

More recently, numerous mice inoculated with blood from pleural fluid taken at autopsy died within a few days with septicemia produced by a bipolar organism that was not identified; the surviving mice showed no change. The organism did not reproduce any manifestations of the disease.

DISCUSSION

The following possibilities were considered in respect to etiology of this disease: (a) infection, (b) degeneration of cardiovascular system, (c) vitamin deficiency, (d) hormonal factors.

The epidemiologic considerations do not suggest an infectious disease and the remarkable sex incidence would also argue against it. Nevertheless, numerous cultural and transmission experiments were made, but without success. Pleuropneumonia-like organisms were not found. Most inoculated animals remained healthy, but in one series 3 of 9 mice injected died with myocarditis, accompanied by *B. typhi murium*, which occurred in the mice purchased for inoculation but not in those of our animal colony. The negative transmission experiments are against causation of this disease by a virus, but it is possible that the proper requisites for successful transmission of the disease have not been met.

The myocardial changes observed were both inflammatory and degenerative in character, and although it is possible that the degenerative changes were primary, the reverse seems more likely. Alterations in the blood vessels of the heart were rare and scant. Involvement of the auricles was more conspicuous than that of the ventricles, and hemorrhage was apparently due to rupture of the auricles. There seems to be no similar disturbance known in man, though in absence of the testicular and pleural hemorrhage the cases would have been designated as isolated (Fiedler's) myocarditis (*cf.* Saphir³).

In these mice there was no generalized hemorrhagic tendency and there are no reasons to suspect vitamin C or vitamin K deficiencies. Deficiency of vitamin B complex, however, should be considered. The cardiac changes in vitamin B₁ (thiamin chloride) deficiency are not well known. They were adequately described by Wenckebach,⁴ who regarded hydropic degeneration as the outstanding microscopic change,

and dilatation of the right heart, particularly that of the pulmonary conus, as the outstanding gross change. It has not been possible to reproduce the anatomic changes of the disease with certainty nor to correlate gross and microscopic changes with the clinical manifestations of the beriberi heart. Recently, Thomas, Mylon and Winternitz⁵ produced myocardial degeneration with subsequent fibrosis by keeping rats on vitamin B₁ deficiency and on a low potassium diet. Observations made by Dock⁶ and by Smith and Furth⁷ suggest that myocardial fibrosis may be a late manifestation of human vitamin B₁ deficiency, but the cardiac changes in mice here described are those of acute or subacute myocarditis and not of myocardial and endocardial fibrosis.

Goettsch and Pappenheimer⁸ reported the production of degeneration of the voluntary muscle of rabbits and guinea pigs when given a diet lacking in vitamin E. More recently, Pappenheimer⁹ has found that the offspring of female mice maintained on a vitamin E-deficient diet showed extensive degeneration of the voluntary muscle fibers, characterized chiefly by hyaline necrosis and infiltration with polymorphonuclear leukocytes. No mention was made of the changes in the heart.

Myocarditis was stated by Lenke and Loewe¹⁰ to be common among mice, but since the occurrence of pleural and testicular hemorrhages was not mentioned, it is not likely that the disease described by them is the same as that studied by us.

Since the disease affected only males during the period of sexual activity, some hormonal factors may be operating. Microscopic examination of the testes, adrenals and hypophysis from several cases, however, showed no conspicuous deviation from normal.

SUMMARY

A disease complex not hitherto reported is described in mice. It is characterized by sudden death with myocarditis and exsanguinating pleuropericardial hemorrhage, accompanied almost invariably by testicular hemorrhage. It is found in about 1.2 per cent of the mice of our colony dying spontaneously and affects healthy, well developed and well nourished male mice, perhaps with rare exceptions. The disease is most common between 10 and 19 months of age, with a peak at 14 months. Epidemiologic observations, transmission experiments and cultural studies failed to support the opinion that the disease is infectious.

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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 23

All sections were stained with hematoxylin and eosin. The magnifications are approximate.

FIG. 1. Heart of mouse Rfe 189 showing advanced myocardial and endocardial infiltration, mainly by polymorphonuclear leukocytes with hemorrhage. $\times 100$.

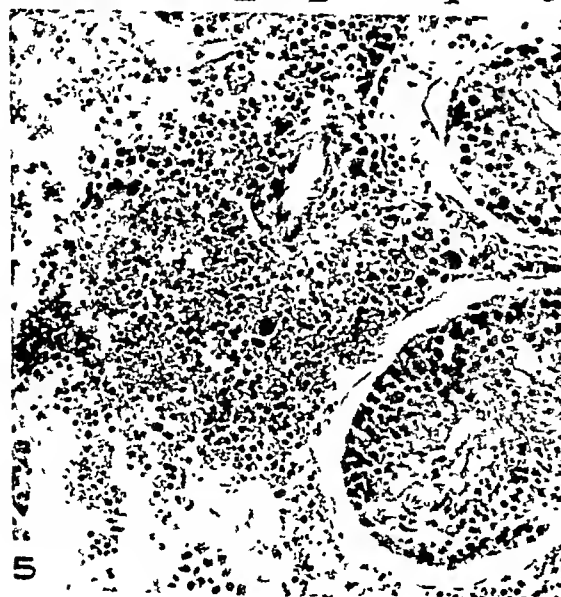
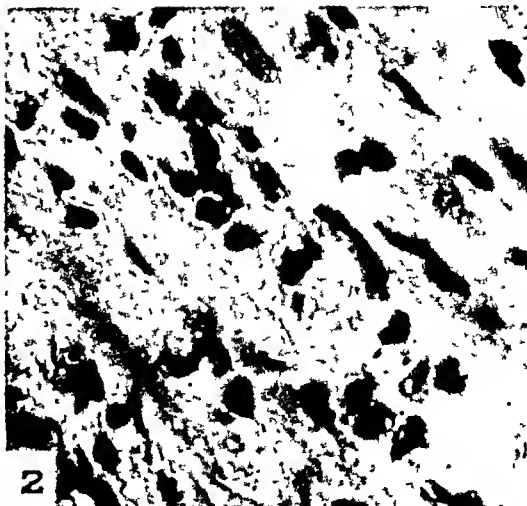
FIG. 2. Higher magnification of the myocardial changes in mouse Rfe 189 showing edema and infiltration by lymphoid cells. $\times 800$.

FIG. 3. Infiltration of the epicardial fat in mouse R.Rff 396, continuous with that of the epicardial and myocardial infiltration. Most cells in this field are polymorphonuclear leukocytes; several are lymphoid cells and monocytes. $\times 800$.

FIG. 4. Myocardial infiltration in the same mouse with predominantly large mononuclear leukocytes and young fibroblastlike cells. $\times 800$.

FIG. 5. Advanced interstitial hemorrhage in the testis of mouse Rfg 342 with large numbers of polymorphonuclear leukocytes among the erythrocytes. $\times 100$.

FIG. 6. Advanced calcification of a medium-sized artery beneath the capsule of the testis of mouse R.Rff 396. On the left side the wall of the artery is very thin and hemorrhage extends beneath the capsule. $\times 100$.





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CALCIFICATION AND PHOSPHATASE *

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In 1923, Robison made the observation that if calcium or barium salts of hexosephosphate are incubated with an extract of bone containing phosphatase, a precipitate of calcium or barium phosphate, respectively, will form. This phenomenon led him to the idea that phosphatase may play a rôle in calcification of bone as it occurs *in vivo*. One year later he found that phosphatase is also present in developing teeth of young animals. Further studies by Martland and Robison showed that phosphatase is not present in cartilage before the appearance of the center of ossification but can be demonstrated in large amounts after the appearance of the center. Fell and Robison cultured embryonic fowl femora *in vitro* and found that the appearance of phosphatase coincides in time with the development of hypertrophied cartilage. Huggins showed that considerable amounts of phosphatase are present in and around bone-producing transplants of bladder mucosa. This finding was confirmed by Regen and Wilkins. The findings mentioned, together with the well known changes in plasma phosphatase level in diseases of the bones (Kay), support the assumption of a close causal relationship between calcification and the presence of phosphatase. Although it has been known since the experiments of Shipley that *in vitro* calcification of calcifiable tissues will occur even in purely inorganic solutions, apparently without phosphatase action, the results of recent studies by Gutman and Gutman indicate that even in the case of inorganic solutions phosphatase plays an important rôle by breaking down hexosephosphoric esters formed locally from glycogen and inorganic phosphate. In conclusion, it seems that phosphatase action is at least one of the mechanisms utilized by the organism to increase the $[Ca^{++}] \times [PO_4^{=}]$ product locally beyond the critical value of 3.3×10^{-6} , which is the solubility product of secondary calcium phosphate at the pH of the blood.

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In 1939 a microtechnical method for the visualization of phosphatase action in tissue sections (Gomori, Takamatsu) became available. Shortly afterward, Ross, and Freeman and McLean published morphological observations on the presence of phosphatase in calcifying trichina cysts and in bone. So far no other histological observations on the relationship between calcification and phosphatase have been published.

In the present paper the relationship between phosphatase and calcification under normal and under a variety of pathological conditions, as shown by a new microtechnical method for the simultaneous visualization of preformed calcium salt deposits and of phosphatase activity, is presented.

MATERIAL AND METHODS

Since calcium salts are removed and phosphatase destroyed by decalcification, all material was embedded and cut without decalcification. This imposed a certain limitation on the material since tissues too hard to be sectioned without decalcification had to be excluded.

Slices of tissues were fixed in 80 per cent alcohol not later than 4 hours after death or removal at operation. Small embryos were fixed *in toto*. The tissues were subsequently dehydrated in 95 per cent and absolute alcohol and embedded in paraffin. Some of the tissues were fixed in ice-cold acetone for studies on acid phosphatase. Paraffin sections were cut from 4 to 6 μ in thickness.

The sections were stained with a modification of my method for the demonstration of phosphatase, devised for the purpose of simultaneous visualization of preformed deposits of calcium salts and of sites of phosphatase activity. The principle of the modification is this: insoluble calcium salts are first demonstrated by transforming them into black cobalt sulfide. The section is subsequently incubated with a solution of calcium glycerophosphate. The calcium phosphate precipitate formed by enzymatic action is stained in a different shade.

The method is as follows:

1. Fix fresh tissues in 80% alcohol, embed in paraffin.
2. Run paraffin sections through xylol and alcohols to distilled water.
3. Treat sections for from 6 to 12 hours with a 2% solution of cobalt acetate. Calcium phosphate and carbonate are transformed into the corresponding cobalt salts. Rinse sections thoroughly in distilled water.
4. Immerse sections for 10 minutes in dilute buffered solution of yellow ammonium sulfide (5 to 6 drops of yellow ammonium sulfide to a Coplin jarful of phosphate or maleate buffer of about pH 7). Unbuffered, strongly alkaline solutions of ammonium sulfide may destroy the enzyme. Cobalt phosphate and carbonate are transformed into black cobalt sulfide. Rinse in water.

5. Incubate sections for from 5 to 6 hours at 37° C. in the following solution:

2% sodium glycerophosphate	25 cc.
2% sodium barbital	25 cc.
Distilled water	50 cc.
2% calcium chloride	5 cc.
2% magnesium sulfate	2 cc.
Chloroform	a few drops

This solution will keep in the ice box for months. Before use add to this mixture a few drops of a 1% solution of some soluble sulfide, such as ammonium or sodium sulfide, in order to depress the solubility of the cobalt sulfide precipitate.

After incubation rinse sections thoroughly in distilled water.

6. Transfer sections for 15 minutes to a 1% solution of lead nitrate. The calcium phosphate precipitate formed at the sites of phosphatase activity is transformed into lead phosphate. Rinse with distilled water.

7. Stain sections in the following mixture for 15 minutes:

0.5% solution of methyl green	2 to 3 parts
0.5% solution of acridine red (Grübler)	1 part

Other brands of acridine red gave results which were much inferior. The staining solution should be not more than 4 weeks old. It should be kept in the ice box when not in use.

8. Differentiate in 95% alcohol, dehydrate in absolute alcohol, clear in xylol, mount in balsam.

Results: preformed deposits of calcium salts, black; sites of phosphatase activity, purplish red; nuclei, green-blue.

OBSERVATIONS

The normal material consisted of numerous embryos of the mouse, rat, guinea-pig, rabbit, dog, pig, chicken and man. The findings were essentially the same in all species.

The earliest sites of phosphatase-positive staining in the skeletal system were the perichondrium of the vertebrae and of the ribs, but in a somewhat later stage the perichondrium of practically all cartilage that in later life develops into bone became positive. The perichondrium contained phosphatase either all around the circumference of the cartilage or in certain patches only. Somewhat later the cartilage itself, especially the portion adjoining the positively-staining perichondrium, showed an intense reaction both in the cell nuclei and in the matrix. It was in these sites of positive phosphatase reaction within the cartilage that the first traces of granular calcification appeared. The deposits of calcium salts were always found in the centers of phosphatase-positive areas. Such areas were about 20 to 50 μ wide. At practically the same stage or somewhat later, cellular strands of connective tissue, representing the anlage of later membranous bones, became intensely positive. In their centers deposits of calcium salts, at first granular but later coalescing to solid strands, made their appearance. Not a single instance of deposition of calcium salts in phosphatase-negative areas could be observed in more than 50 embryos

of various species and ages. Phosphatase-positive cartilage or connective tissue without any signs of calcification was seen quite often, but since no serial sections were made it is impossible to tell whether these positive areas did or did not contain centers of calcification at some other level.

In the bone tissue the osteoblasts remained positive for some time but soon lost their positivity in the more central, older portions of the bone, whereas in all areas where apposition was active, *i.e.*, under the periosteum and in the epiphyses, the osteoblasts were always strongly positive.

Teeth were examined only in a few rat embryos. Intense phosphatase reaction was obtained in the stratum intermedium of the enamel organ and also in the connective tissue of the pulp, especially in a layer immediately subjacent to the odontoblasts in regions where calcified dentin was present. The ameloblasts and the odontoblasts were negative or, in some cases, showed traces of positive staining.

It seems to be appropriate to include here the observations on one case of experimental rickets in a rat, although rickets is not a normal condition. The proximal tibial epiphysis was examined. Phosphatase was found to be present only in the hypertrophied zone of cartilage, while the remainder of the epiphyseal plate was negative. No calcification was present in the cartilage matrix. The osteoid borders of the bone trabeculae were negative for phosphatase but the osteoblasts lining them were positive. These observations are in full agreement with those of Freeman and McLean.

Bone formation under the influence of transplants of bladder epithelium was studied in 8 dogs, 4 guinea-pigs and 4 rabbits. Six pieces of the epithelium of the urinary bladder were transplanted in each animal to both rectus sheaths (three on each side) according to the technic of Huggins. They were spread as flatly as possible, the surface of the epithelium facing the skin. In addition, in 4 dogs small pieces of bladder epithelium were buried in the parenchyma of the liver and spleen and between the muscular layers of the stomach. The transplants were removed at regular intervals over a period of 10 weeks.

Epithelial cyst formation was observed in from 6 to 10 days at the sites of all transplants in dogs and in guinea-pigs and in 1 rabbit. In 3 rabbits the transplants disappeared.

Up to the point of completed cyst formation there was no difference between the histological pictures of the fascial and the intraparenchymatous transplants. In all transplants the epithelium retained its originally phosphatase-positive staining in the transplanted as well as in the regenerated portions. However, after the stage of cyst formation

the events took an entirely different course in transplants to the liver, the spleen and the stomach, on the one hand, and in transplants to the fascia, on the other hand.

In the liver, the spleen and in the wall of the stomach the cysts grew to a certain size, became rather distended, the epithelium became flattened and the entire structure was surrounded by a thick capsule of connective tissue. The epithelial lining remained positive throughout. No phosphatase was seen in the capsule.

In the fascial transplants on the 6th day the connective tissue subjacent to the newly formed epithelium showed a few scattered, strongly phosphatase-positive fibroblasts. In the next few days the number of these phosphatase-positive cells rapidly increased, and at the same time the fibrillar ground substance between them also became diffusely positive. By the 9th and 10th day a thick plaque of coarsely fibrillar, osteoidlike, intensely phosphatase-positive connective tissue was found under the newly formed epithelium. The thickness of the plaque was from 20 to 200 μ . It was not in immediate contact with the epithelium but was separated from it by an intermediate phosphatase-negative layer of connective tissue, about 20 μ in thickness. No reaction of the type described was seen under the old surviving epithelium of the transplant. By the 13th to the 20th day some of the densest strands in the centers of phosphatase-positive areas showed a granular deposit of calcium salts. The calcified areas rapidly extended and ramified. By the end of the 1st month well developed bone with hemopoietic marrow was seen in all transplants. The bone was surrounded by a wide zone of a rather cellular, intensely phosphatase-positive connective tissue, simulating the picture seen in embryonic osteogenesis. The entire process was essentially the same in dogs, guinea-pigs and in the rabbit.

As mentioned, bone formed only in the connective tissue underlying the newly formed epithelium. This is in complete agreement with the observations of Huggins.

The pathological material will be divided in three groups: (1) bone tumors; (2) tuberculosis; (3) calcification in hyaline connective tissue.

1. Bone Tumors

Four cases of bone-forming osteogenic sarcomas were observed. The pictures they presented were closely similar. All of the tumors were very strongly positive for phosphatase, especially in their most cellular, peripheral portions. Both the cells themselves and the fibrillar intercellular substance partook in the reaction. Granular deposits of

calcium salts were seen in strands of connective tissue within the positive areas. These deposits gradually coalesced until the entire stroma was outlined in black, with clear cells encased in the meshes. Two of the tumors had a marked tendency to sclerosis. In the sclerosing areas the reaction was far less strongly positive than in the cellular ones, except for groups of strongly positive cells growing within the vessels. Highly sclerotic, almost acellular areas were found to be entirely negative.

In addition to the cases mentioned, one case of osteoplastic metastasis of a cancer of the breast was observed. The tumor cells themselves were entirely negative for phosphatase, whereas the stroma was strongly positive, with extensive calcification and bone formation in the centers of positive areas.

Two giant cell tumors and two fibrosarcomas of bone, without new bone formation, were entirely negative for phosphatase, except for a few phosphatase-positive capillaries.

2. *Tuberculosis*

The material consisted of 42 rabbits, 58 guinea-pigs and 3 human cases. The rabbits were inoculated with 0.2 to 2 mg. of the culture of a bovine strain subcutaneously or intratracheally, the guinea-pigs with 2 mg. of a human strain subcutaneously. The animals were killed at regular intervals or allowed to die over a period of 55 weeks.

Observations on the three species will be given separately.

Rabbit. In the normal rabbit's lung phosphatase occurs in variable amounts in three different sites: first, the lining of the alveoli; second, the endothelium of blood vessels; third, in leukocytes and lymphocytes. The rabbit's neutrophils ("specials") are by no means as uniformly positive for phosphatase as those of the guinea-pig. Only a certain percentage of them are positive, and the ratio of positive to negative is usually much lower within the blood vessels than it is within the tissues. There is no morphological difference between the positive and negative cells; in fact, the two kinds are entirely indistinguishable from each other without the use of the phosphatase reaction. For the time being it is impossible to tell what factors control the number of positive and negative leukocytes. The above statements apply also to the lymphocytes. Within the blood stream most of them are negative, whereas in lymphocytic infiltrates the positive type prevails.

Early tuberculous granulation tissue without signs of necrosis was found to be entirely negative for phosphatase except for that contained in leukocytes and lymphocytes. As soon as necrosis set in phosphatase

appeared in the centers of the necrotic areas, first in the shape of an irregular, coarse network, later coalescing to a uniform, more or less rounded area of very intense phosphatase reaction. The enzyme was not carried in leukocytes since it appeared in all necrotic tubercles, regardless of whether they did or did not contain leukocytes. The positivity of the areas of necrosis did not last long as phosphatase soon disappeared from the centers, only to appear in the area of fresh necrosis in the peripheral zone of spread. In this way ever receding and enlarging rings of phosphatase-positive staining occupied the inner, necrotic area of the zone of spread. If the process came to a standstill, phosphatase disappeared altogether.

The intensity of phosphatase-positive staining in the early necroses was so high that it is impossible to account for the amount of enzyme present in them from purely local sources. As mentioned, phosphatase is not carried in by leukocytes. The synthesis of such a highly specific enzyme by a necrotic mass is improbable. The most probable explanation seems to be the adsorption of the enzyme from the blood and from the tissue fluids by the fresh necrotic material, owing to some physico-chemical property of the latter at a certain stage of development. At a later stage this property is lost and consequently the enzyme is eluted from the lesion. It should be mentioned here that in two rabbits necrosis of the lung and liver were produced by the intraparenchymatous injection of alcohol. No phosphatase was found in areas of necrosis produced by this method.

The changes mentioned were remarkably uniform in the pulmonary lesions of all animals except two. In the latter cases phosphatase failed to appear in the necrotic areas. The phosphatase picture of lesions in other organs was far less regular. Most areas of necrosis were found to be free of phosphatase, regardless of their age; and even when phosphatase was present, the reaction was much fainter than in the lesions of the lungs.

Calcification was observed in 15 cases out of 42. It involved pulmonary lesions only, although extensive tuberculosis of the spleen, liver and kidneys was present in many of the cases. The earliest date at which calcification could be demonstrated was 38 days after inoculation (average: 101 days).

If calcification took place, its earliest traces invariably occurred in the center of some phosphatase-positive area. Calcification did not alter the peripheral shift of phosphatase as described. Phosphatase receded in the shape of a ring from the central calcification in the typical way. In the peripheral positive area new centers of calcification often developed which, if they coalesced, led to the formation of im-

perfect concentric calcareous shells. This pattern of concentric rings is well known from x-ray studies on human tuberculosis.

Guinea-Pig. The normal lung of the guinea-pig does not contain any phosphatase at all, except in the scattered leukocytes which are constantly and strongly positive in this species and in the epithelial lining of some of the bronchi.

Tuberculous granulation tissue as well as areas of necrosis were entirely free of phosphatase, except for the leukocytes. In my series of guinea-pigs the lesions were of a markedly exudative character and the tubercles in practically all cases were densely infiltrated by leukocytes. The latter often formed strongly phosphatase-positive abscesses in the centers of the lesions. After the breakdown of the leukocytes the enzyme diffused in all directions. All the phosphatase in guinea-pig tubercles comes from leukocytes. These data are in complete agreement with those presented by Takeuchi and Takamatsu.

Calcification was observed in only 3 cases out of 58. It involved both pulmonary and splenic lesions. The earliest date at which calcification could be found was 55 days after inoculation. It always occurred in the centers of necrotic areas. Phosphatase could be seen around some of the calcified centers, but owing to the small number of cases no further conclusions can be drawn.

Man. The normal human lung contains extremely variable amounts of phosphatase in three sites: first, in the lining of the alveoli; second, in the endothelial lining of capillaries; third, in the epithelium of the bronchi. Human leukocytes are free from phosphatase.

The number of human cases observed being only three, no definite conclusions can be drawn as to the phosphatase picture in human tuberculosis. Tuberculous granulation tissue was found to contain no phosphatase. There was a marked perifocal intensification of the reaction in the alveolar lining. In one case the outer fibrous wall of a cavity was found to be positive. Takeuchi and Takamatsu made practically the same observations. Calcification was observed in one old, encapsulated lesion, with no trace of phosphatase around it

3. *Calcification of Hyaline Connective Tissue*

This group includes 6 examples of early arteriosclerosis of the aorta; 2 of sclerosing and calcifying pyelonephritis in human beings and 1 in a rabbit; 2 calcifying goiters and 1 calcifying islet cell tumor of the pancreas. In all of these, granular calcium salt deposits were observed in very dense, almost acellular, connective tissue. No trace of phosphatase was found around any of the granules of calcification.

As mentioned, sections of practically all specimens were also stained

by my technic for the demonstration of acid phosphatase. In no instance could the presence of this enzyme in or around calcareous deposits be demonstrated.

COMMENT

Calcification of living or recently necrosed tissue seems to start invariably in the centers of intensely phosphatase-positive areas. On the other hand, calcification of sclerosed, hyaline connective tissue apparently can and does occur without phosphatase action. Although it is possible that the latter type of calcification may be initiated by phosphatase action followed by a rapid disappearance of the enzyme, the fact that phosphatase was not found in a single instance around deposits of calcium in hyaline connective tissue is against such an assumption. Obviously there are other mechanisms besides phosphatase action capable of raising the $[Ca^{++}] \times [PO_4^=]$ product beyond the critical level. The results of Ross clearly show that under certain conditions tissues may calcify far below the $[Ca^{++}] \times [PO_4^=]$ value of 3.3×10^{-6} , required for the calcification of bone. The nature of the mechanism involved in calcification at low $[Ca^{++}] \times [PO_4^=]$ products is not understood at present. One possibility, unexamined so far, would be the presence of some protein with a higher dissociation constant than that of plasma proteins, resulting in a higher local concentration of calcium ions at the same level of total calcium.

An interesting problem is the source of phosphatase in the connective tissue around transplants of bladder epithelium. The two possibilities considered are: (1) simple diffusion of the enzyme (and, according to Huggins, of calcium and phosphate) through the thin layer of newly formed epithelium into the surrounding tissue, and (2) specific inductive power of the bladder epithelium on certain kinds of connective tissue, resulting in the proliferation of phosphatase-producing fibrocytes. The theory of simple diffusion does not explain the fact that calcification and bone formation is regularly produced at certain sites (fascia), while consistently negative results are obtained at other sites (liver, spleen, stomach), unless the presence of an antiphosphatase in these latter organs is hypothecated. On the other hand, the theory of specific induction would fit well into proved facts of embryology. Gruenwald published striking instances of strictly region-bound inducibility of the mesenchyme by the growing wolffian duct.

An attempt was made to solve this problem by transplanting other, normally phosphatase-positive, kinds of epithelium to the fascia and by observing whether or not phosphatase and bone formation would

appear around them. In 3 dogs pieces of duodenal mucosa and in 5 more dogs and in 3 guinea-pigs pieces of the epididymis (finely minced to provide for a large surface of contact) were transplanted to the rectus sheath. The transplants of duodenal mucosa were complete failures. They all disappeared within 3 weeks. The transplants of epididymis took well and grew considerably in 10 weeks. Many epithelium-lined cysts were observed. Although the epithelium in many places retained fully its phosphatase-positive character, no phosphatase and no bone formation were observed in any of the transplants. The results of these experiments seem to support the theory of specific induction rather than that of diffusion. Induction of bone formation by bladder epithelium seems to be a favorable case in which it is possible to trace the induction mechanism one step farther back than in most other instances.

SUMMARY

A new microtechnical method for the simultaneous demonstration of preformed calcium salt deposits and of sites of phosphatase activity is presented, together with observations made with this method on normal and pathological calcification.

Calcification of living or recently necrosed tissues seems invariably to involve phosphatase activity. On the other hand, calcification of hyaline connective tissue occurs without any phosphatase action. An attempt is made to explain the difference between the mechanisms of these two types of calcification.

Acid phosphatase plays no rôle in calcification.

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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 24

Calcifications, black; sites of phosphatase activity, purplish red; nuclei and ground substance of cartilage, green-blue.

FIG. 1. Parasagittal section of a rat embryo, 18 mm. long, showing processes of vertebrae. Positive reaction in the perichondrium. $\times 36$.

FIG. 2. Lower incisor of a newborn rat. $\times 30$.

FIG. 3. Bone-producing transplant of bladder mucosa. Positive reaction in the basal layer of the epithelium and in a thick strand of connective tissue in the upper right part of the field. The bone is surrounded by a wide zone of positive reaction. $\times 36$.

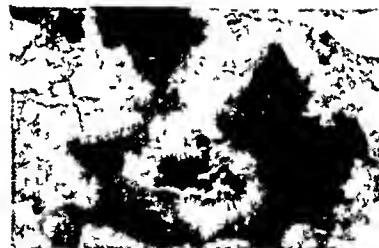
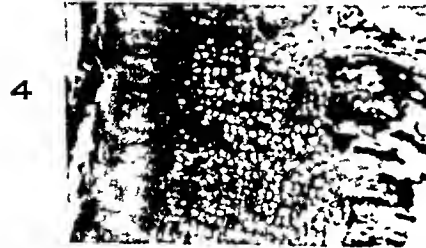
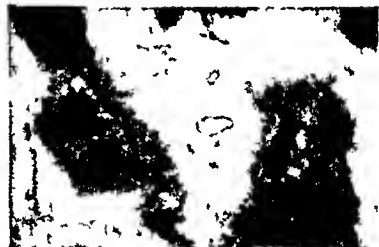
FIG. 4. Upper tibial epiphysis of a rachitic rat. The zone of hypertrophied cartilage is strongly positive for phosphatase. $\times 48$.

FIG. 5. Osteogenic sarcoma of the tibia. The tumor is strongly positive, the surrounding connective tissue is negative. $\times 36$.

FIG. 6. Phosphatase reaction in the center of an early necrotic tubercle in the rabbit's lung. $\times 36$.

FIG. 7. Calcification in centers of phosphatase-positive areas (rabbit's lung). $\times 36$.

FIG. 8. Peripheral recession of phosphatase reaction with secondary calcification at the periphery (rabbit's lung). $\times 30$.



Gomori

Calcification and Phosphatase

THE NATURE OF THE RENAL LESION WITH THE SULFONAMIDES AND ITS PREVENTION WITH UREA *

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A review of the reported renal changes exhibited by the various sulfonamide compounds in animals and man reveals, with minor differences, a constant pathologic picture.¹⁻¹⁰ The cause of these changes has not been clearly demonstrated and the relative rôles of primary chemical injury and of trauma secondary to mechanical precipitation have not been adequately evaluated.¹¹⁻¹³

In a study of the *in vitro* solubility of acetylsulfathiazole and acetylsulfapyridine in urine, Curtis and Sobin¹⁴ found that at a given pH solubility increased in proportion to the specific gravity of the urine. From both theoretical considerations and experimental data it was pointed out that the factor responsible for the increase in solubility of these materials in more concentrated urines was probably an increasing amount of urea. It was at once apparent that what was true in the test tube might also be true in the animal body. The *in vivo* use of urea might, therefore, enable a distinction to be drawn between the primary nephrotoxic properties of these drugs and mechanical irritative effects resulting from their intrarenal precipitation.

METHOD

Analysis of precipitated material found in the renal tract when the sulfonamide drugs are administered over an extended period to man and animals has shown a high percentage of acetylated derivatives.¹⁵⁻¹⁹ Oral administration of these acetylated drugs is unsatisfactory because of insolubility and erratic absorption. Accordingly, sodium acetylsulfapyridine was prepared by the method of Marshall, Bratton and Litchfield²⁰ for the sodium sulfonamides.†

Forty-eight adult white male rats were divided into four groups of 12 and treated as follows: groups 1 and 2 were each given 1 mg. of sodium acetylsulfapyridine per gram of body weight, but in addition group 2 received 5 mg. of urea per gram of body weight. Groups 3 and 4 received 3 mg. of sodium acetylsulfapyridine per gram of body weight and group 4 received in addition 10 mg. of urea per gram of body weight. Untreated rats of the same age were used as controls.

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† Attempts to prepare crystalline sodium acetylsulfathiazole by this method were unsuccessful and sodium acetylsulfapyridine alone was used in these experiments.

The drugs were administered in solution through a stomach tube. The volume of solution of sodium acetylsulfapyridine was calculated on the basis of rat weight and the urea was added. Animals were treated over periods of 7 to 14 days; some were treated daily and others intermittently. Throughout the period of observation the daily water intake and the weight were noted.

Tissue sections were prepared in the usual manner with the precaution that previous to embedding in paraffin all solutions were saturated with acetylsulfapyridine. This included the formaldehyde fixative and the various dehydrating and clearing agents. Such a procedure eliminated the necessity of a rapid and special method for the demonstration of intrarenal sulfonamides^{3, 21} (Fig. 1). Serial sections were made of both kidneys, and a systematic examination was carried out.

OBSERVATIONS

Throughout the progress of the study it was noted that animals treated with sodium acetylsulfapyridine alone, frequently presented a

TABLE I
Fluid Intake of a 10-Day Experiment
(computed as cc. per gm. of rat per day)

	Day										
	0	1	2	3	4	5	6	7	8	9	10
Group A*	0.27	0.20	0.18	0.22	0.23	0.21	0.16	0.28	0.13	0.24	0.25
Group B†	0.25	0.31	0.25	0.17	0.20	0.18	0.14	0.29	0.19	0.18	0.37
Group C‡	0.24	0.36	0.17	0.20	0.19	0.17	0.12	0.24	0.19	?	0.12

* Sodium acetylsulfapyridine (3 mg. per gm. of rat per day) without urea. (Animals receiving 5 mg. of urea in addition to 1 mg. of sodium acetylsulfapyridine per gm. of rat showed no increased water intake over animals not receiving urea.)

† Sodium acetylsulfapyridine (3 mg. per gm. of rat per day) with urea (10 mg. per gm. of rat per day).

‡ Controls.

hematuria which was not constant from day to day. Hematuria was not encountered in any animal receiving urea along with sodium acetylsulfapyridine. The animals not receiving urea frequently appeared ill, listless and took their food poorly. This was not true of the urea group.

A study of the daily water intake (computed as cc. per gram of rat per day) showed no increase in the urea-treated animals (Table I). Inasmuch as the interval from the beginning to the end of the experiment was sufficient to allow for water balance, the urea effect cannot be attributed to diuresis.

The impression gained from the hematuria was confirmed upon

examination of the kidney sections. Two types of foreign material were found in many of the kidney sections of the animals treated with sodium acetylsulfapyridine alone: intrarenal, nonstaining crystals (Fig. 1) and bluish staining (with hematoxylin) intratubular masses (Fig. 2). In animals receiving 1 mg. per gram of body weight of the drug, calculi * were noted in slightly less than half of the kidney sections; in those receiving 3 mg. per gram of body weight of the drug, they were noted in about 90 per cent of the cases. Calculi were not found in the kidney sections of any animal in the urea-treated groups.

A low-power magnification of sagittal kidney sections showed most of the calculi to be at the corticomedullary junction; however, deposition in other areas of both cortex and medulla was observed. Precise localization of calculi was difficult. Under higher magnification many were found within Henle's loops, convoluted tubules and collecting ducts, but it was impossible to differentiate proximal from distal tubules in the sections studied. Antopol⁴ and Lehr and Antopol⁹ were able to recognize distal convoluted tubules as the site of calcification.

In order to determine the composition of the intratubular blue-staining masses, attempts were made to stain the acetylsulfonamide compounds. With hematoxylin, silver stains (Fig. 1) and fat stains it was impossible to stain acetylsulfapyridine. The basophilic nature of the calculi with hematoxylin further suggested that some substance other than acetylsulfapyridine was present. Use of von Kossa's stain for calcium indicated that this was true and that the calcium content was high (Fig. 3). Calcium deposition was seen to occur in at least two ways: as small calcific deposits (Fig. 4) and by lamellation upon these small foci (Fig. 2).

The mechanism of calculus formation is important. In the dosage used it took 9 or 10 days before calcium could be found in mass deposits. In sections taken from animals treated for 10 days and then sacrificed, liberal amounts of calcium were noted (Figs. 2 and 3). Animals sacrificed after 7 days of treatment showed only focal areas of severe tubular degeneration (Fig. 5). These tubular epithelial cells were markedly edematous with disappearance of former cell margins and the formation of a granular protoplasmic mass. The nuclei were swollen, and fragmented nuclear material was recognizable. These

* A distinction must be made between the various terms applied to foreign material found in the renal tract with sulfonamide therapy. *Concretion*, *calculus*, *urolith* and *stone* have been used indiscriminately. We reserve *calculus* for those bodies with a mineral content, especially calcium, and *concretion* for any solid body. *Urolith* and *stone* by derivation imply a mineral or calcium content. Precipitated sulfonamides will be referred to as such, or as concretions.

areas took a bluish cast with hematoxylin and eosin stains, possibly due to diffuse chromatin. Many such areas were found in tissues showing abundant concretions. In the latter tissues, routine stains showed tiny basophilic areas and corresponding silver stains showed foci of calcium deposition (Fig. 6). These areas of focal calcium deposition also showed iron deposition by the Prussian-blue reaction. In the tissues of animals treated only 7 days these areas of focal necrosis showed no calcium deposition. Areas of focal regressive change were not encountered in urea-treated animals or in normal controls.

Fat stains did not demonstrate degenerative fatty infiltration in either severely injured kidneys with focal necrosis and other regressive changes or in the kidneys of animals protected with urea.

Distinction must be made between areas of tubular necrosis with secondary calcification as described above and areas of tubular damage from pressure necrosis by calculi already present (Fig. 7). This latter type of calculus was formed by deposition of calcium salts upon necrotic epithelial cells free in the tubules. The epithelium of the tubule was seen to be intact except where the foreign body was in contact with it. In some sections a leukocytic response was noted at the areas of contact of calculus and epithelium. Although calcification upon an organic nidus can occur either *in situ* or free on necrotic epithelial cells, the significance of such calcification does not differ in either case.

Renal changes other than areas of focal regressive change and calculus formation were similar to those adequately described and illustrated by others and will not be considered in detail here. Cloudy swelling, focal and generalized interstitial cellular infiltration, pyelitis and dilatation of the whole nephron, including compression of the glomerular tuft and intracapsular deposition of foreign material, have been seen in varying degree only in the animals treated with acetylsulfapyridine. Thickening of the basement membrane of Bowman's capsule, with foreign material in the capsular space, has been noted in human tissues;^{7,9} minor changes of this type have also been seen in this material.

DISCUSSION

The prevention of the renal calculi resulting from administration of sodium acetylsulfapyridine by means of simultaneously administering urea depends upon a solvent effect of urea on both free²² and acetylated sulfonamide compounds.¹⁴ That such solvent effect occurs in both test tube and animal body is some evidence of its specificity.

Originally, the possibility of calcium deposition upon precipitated intratubular sulfonamide was suggested by Antopol and Robinson.²³

Recently this view has been again expressed by Lederer and Rosenblatt⁸ from a review of human material. That intrarenal calcium deposition did occur with administration of free and acetylated sulfonamide compounds was subsequently demonstrated by Antopol and co-workers.^{3-5, 9, 11} They have termed this lesion *calcifying nephrosis*. Details of the pathogenesis and significance of such calcification have not been previously reported. With the dosage used in these experiments it takes about 10 days for calcification to occur. Antopol, Lehr, Churg and Sprinz⁵ have reported calcification in 24 hours when massive intrarenal precipitation of sulfonamides occurs as a result of parenteral therapy with the soluble sodium salts.

In a study of the renal pathology resulting from mercuric chloride poisoning, Harmon²⁴ reported changes similar to those described above. Regressive changes ranging from severe parenchymatous degeneration to necrosis of the tubular epithelium were distributed from proximal convoluted tubules to collecting tubules. Glomerular changes were not found. Two types of cell death were present: (1) simple coagulation necrosis without edema, with pyknosis or disappearance of nuclei, attributed to the primary nephrotoxic properties of mercury; and (2) marked cellular swelling with large vesicular fragmented nuclei, possibly associated with acid intoxication and the resulting anuria. Calcification of the necrotic epithelium occurred in free masses in the tubular lumen and in the tubular lining cells *in situ*. No calcification of apparently living cells was found. Photographs showing calcium deposition as a result of mercurial intoxication are indistinguishable from those seen with sodium acetylsulfapyridine.

Smetana²⁵ has designated the renal pathology resulting from carbon tetrachloride intoxication as *nephrosis* characterized by "distention of the spaces of Bowman with albuminous precipitate, with swelling of the lining cells, swelling and vacuolation of the cells of the proximal convoluted tubules, degeneration and necrosis of the cells of the distal convoluted tubules and those of the loops of Henle, with desquamation, and by the presence of granular, hyaline and cellular casts in the tubules, with plugging of their lumina. Concretions are present whose nature and significance are obscure." These concretions were either yellow-green or intense blue with hematoxylin and eosin stains, and stained black with von Kossa's stain. Although Smetana believed that calcification of necrotic tubular cells, as commonly observed in bichloride of mercury poisoning, is not a feature of carbon tetrachloride poisoning, the morphology and staining qualities of the concretions which he described leave no doubt that deposition of calcium had occurred.

Animal experimentation indicates that the renal injury in experimental bismuth intoxication is not dissimilar from that described for mercurial poisoning.²⁶ Abundant calcium in the kidneys of rabbits treated with bismuth has been recently reported by Kroll, Arens, Mesirrow, Strauss and Necheles.²⁷

In the absence of intrarenal precipitation of free and acetylsulfonamide compounds, significant alterations of renal architecture are not found. This suggests that a direct relationship exists between calculus formation and precipitated sulfonamides, even though crystalline sulfonamide is not recognizable in these calcific masses. Our interest in the renal changes with the heavy metals and carbon tetrachloride is in the fact that chemical injury, with associated regressive changes leading to epithelial tissue death, may be followed by deposition of calcium upon the nonviable tissues as a focus. We believe that the pathogenesis of renal calcification observed with sulfonamide drugs is by a similar mechanism and is a general response of the kidney to injury. Mechanical irritation occurs as a result of precipitation of these materials; the resulting tissue injury may be severe enough to produce local necrosis. It is upon these areas of necrotic tissue that calcium deposition occurs.

Reference has already been made to reports on the renal changes aside from calculus formation.¹⁻¹⁰ The distended tubular system has been recognized as hydronephrotic; the type and degree of such a process should depend on the amount of precipitated sulfonamide (free and acetylated) blocking the tubular system. Comparable lesions have been produced by ligation of the ureteropelvic junction.²⁸ Interstitial inflammatory changes and thickening of the parietal layer of Bowman's membrane have also been observed with this same procedure. As yet it has not been demonstrated that these changes noted with sulfonamide therapy are independent of tubular block and hydronephrosis *per se*. Focal and diffuse parenchymatous degeneration is adequately explained by mechanical trauma, and chemical trauma has not been established.

Of additional importance are the renal changes that result from administration of amounts of sulfonamide drug below that which produce intrarenal crystal formation by microscopic examination.¹¹⁻¹³ These changes are similar to those described above. Prior to the utilization of urea in preventing precipitation of sulfonamide in the renal tract, this method of minimal dosage was the only one available to determine chemical nephrotoxic properties of these drugs. There is no positive evidence that crystal formation has not occurred with even the relatively small amounts of these drugs necessary to produce renal

lesions. It is certain that simultaneous administration of urea does prevent the pathologic changes in the kidney exhibited by these drugs.

The extremely rare renal lesions with sulfanilamide and neoprontosil have been associated with demonstrable crystalline masses in the kidney.²⁹ The recent report of Lehr and Antopol⁹ on renal complications with sulfadiazine stated that "it is the physical factor of poor solubility, as generally created by acetylation, rather than the chemical change of the compound which accounts for the kidney damage." We subscribe to this view.

Focal necrosis has been seen in human material that presented the clinical syndrome of sulfathiazole toxicity. No counterpart of this symptom-complex has as yet been encountered in animals and it is doubtful that the mechanism of production of lesions is similar.

A consideration of the differential function of the various parts of the nephron, as outlined by Edwards,³⁰ may explain the reported differences in the site of precipitation of the different sulfonamides.¹⁶ It may further explain the susceptibility to renal complications from the use of sulfonamides in patients with previous renal injury.

CONCLUSIONS

1. Intrarenal foreign material following sulfonamide drug therapy is of two types: (1) precipitated sulfonamide and its acetylated products; (2) cellular débris, with calcium and iron deposition around or on this material.

2. Urea simultaneously administered with sodium acetylsulfapyridine will prevent the precipitation of sulfonamides and the formation of renal calculi in rats.

3. The action of urea is independent of a diuretic effect and depends upon a specific solvent effect on acetylsulfapyridine.

4. The nephrotoxic properties of acetylsulfapyridine are mechanical in nature, and result from precipitation of the drug in the renal tract.

5. Calcification in the kidney and the resultant calculus formation in sulfonamide-treated animals is dependent upon local tissue damage and the secondary deposition of calcium and iron upon focal, nonviable structures.

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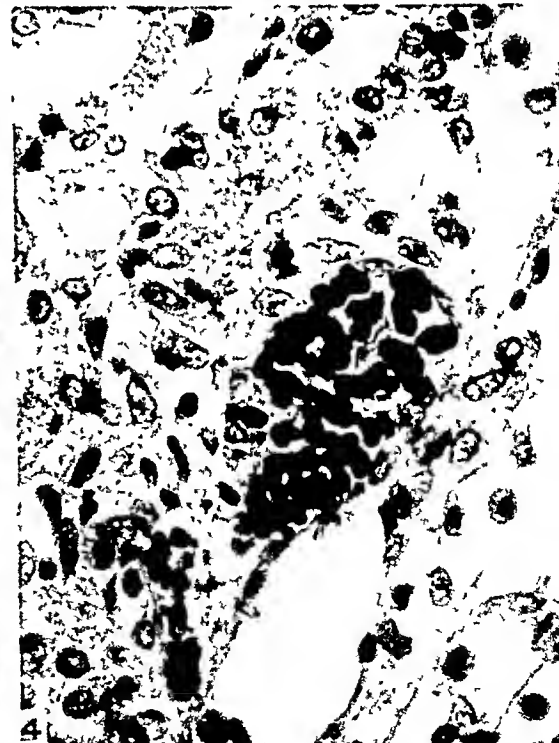
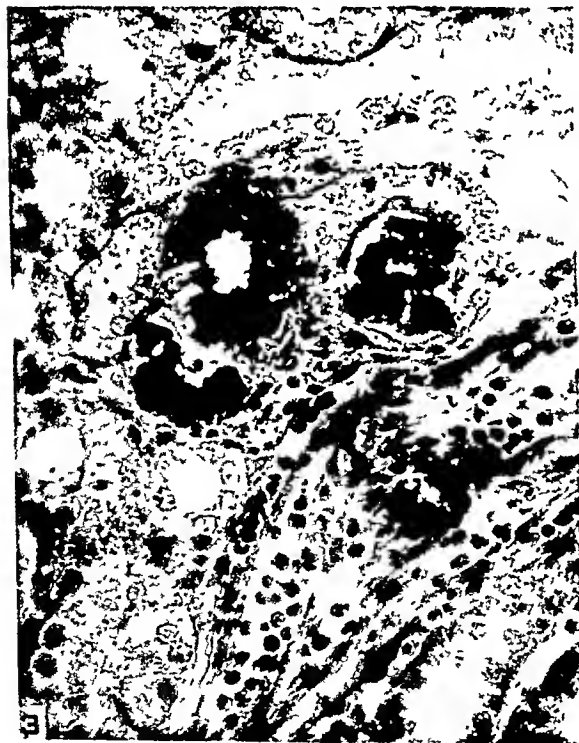
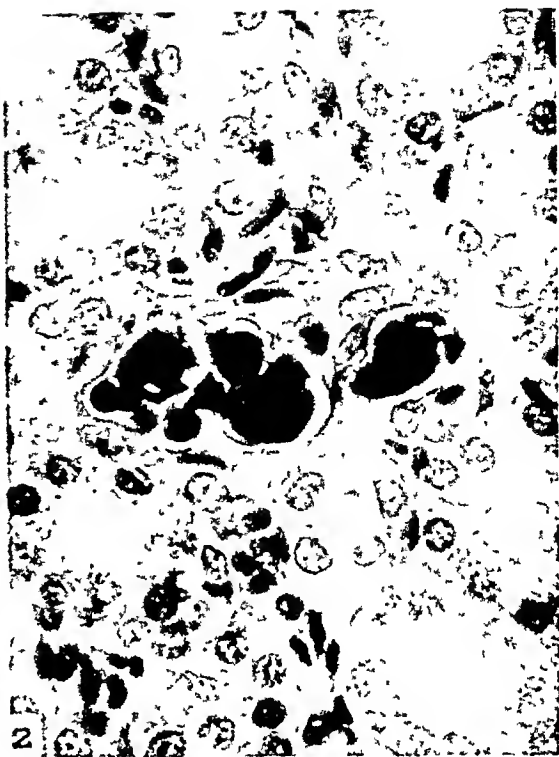
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 25

- FIG. 1. Cortex of kidney of rat showing sheaths of nonstaining acetylsulfapyridine crystals in collecting ducts. Packed erythrocytes surround the crystals in the central tubule; leukocytes can be seen at the lower border of the packed cells. Intratubular leukocytes can be more clearly seen in the sheath at the lower right, as well as an early interstitial leukocytic response at the contact area of the sheath and the injured duct wall. Masson and von Kossa's stains. $\times 145$.
- FIG. 2. Intratubular blue-staining masses in cortex of kidney of rat. There is complete absence of tubular epithelium. Calculi show concentric deposition of calcific material. The tubules surrounding the concretions are intact. Hematoxylin and eosin stain. $\times 575$.
- FIG. 3. Calcium deposition in intratubular concretions. Preparation as in Figure 1. $\times 325$.
- FIG. 4. Corticomedullary junction of kidney of rat with multiple small foci of calcium deposition in an area of tubular necrosis. Preparation as in Figure 2. $\times 510$.

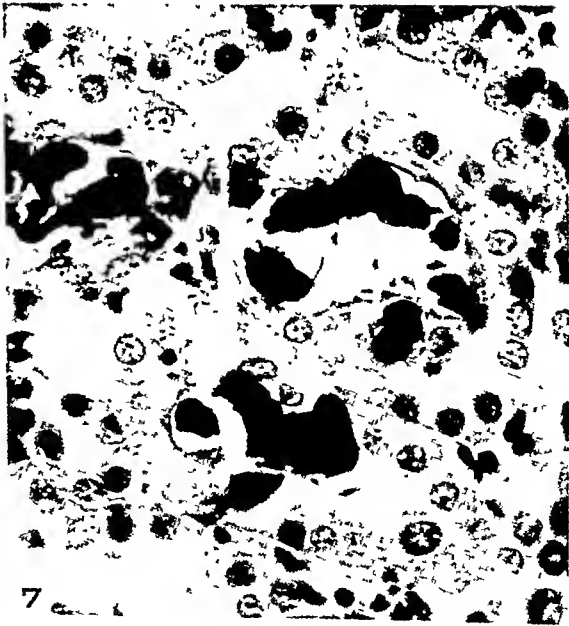
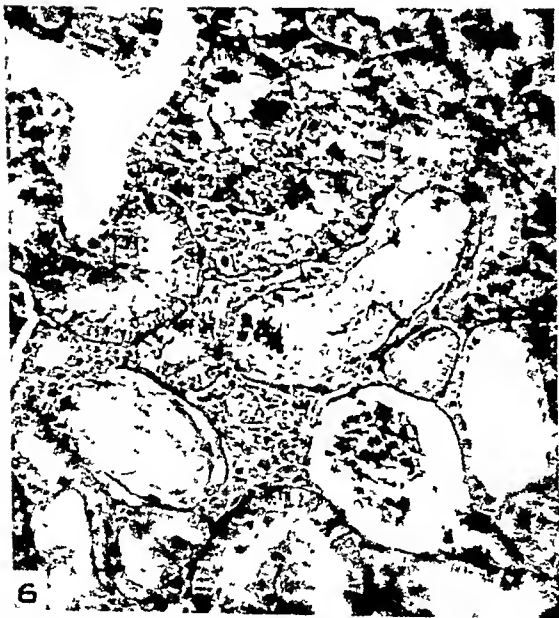
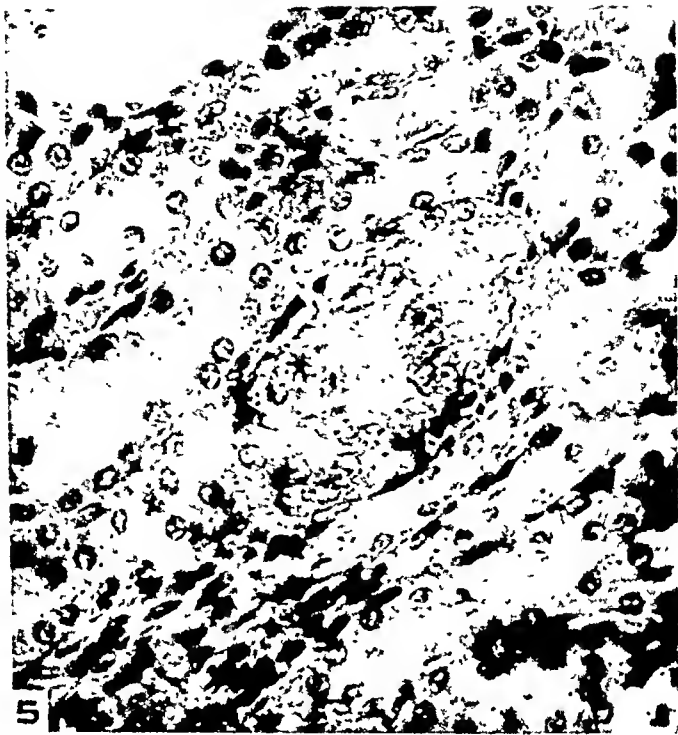


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Renal Lesions with the Sulfonamides

PLATE 26

- FIG. 5. Focal necrosis of the tubular epithelium. This area takes a bluish stain with hematoxylin and eosin. Swelling of nuclei, loss of cell boundaries and granularity of cytoplasm are seen. Hematoxylin and eosin stain. $\times 400$.
- FIG. 6. Early calcium deposition in an area of focal necrosis. There is a severe degenerative change of the tubular epithelium with scattered early foci of calcium deposition. Masson and von Kossa's stains. $\times 210$.
- FIG. 7. Tubular injury secondary to concretion formation. There is a loss of continuity of tubular epithelium at the area of contact of preformed intratubular concretions with the tubular wall. The epithelium is otherwise intact. Hematoxylin and eosin stain. $\times 435$.



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Renal Lesions with the Sulfonamides

ERYTHROPHAGOCYTOSIS AND HEMOSIDEROSIS IN THE LIVER AND SPLEEN IN SICKLE CELL DISEASE *

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Abnormal destruction of erythrocytes by the cells of the reticulo-endothelial system is characteristic of many hemolytic anemias. However, these cells seldom exhibit morphologic evidence of erythrophagocytosis. It has been seen occasionally in pernicious anemia, hemolytic jaundice, sickle cell anemia, icterus gravis neonatorum and Weil's disease. The degree to which this erythrophagocytosis is apparent differs in various parts of the reticulo-endothelial system. The spleen, as a rule, shows very little evidence of this process.¹

The pathologic changes of the different organs in sickle cell anemia have been repeatedly summarized.²⁻⁶ It has been stated that the Kupfer cells in the liver frequently exhibit phagocytized red blood cells.^{2,5,7} The changes in the spleen also have been thoroughly investigated.⁸ The details, however, as to the frequency and extent of these processes are not yet established.

The case of sickle cell disease herein reported is illustrative of a disproportional degree of erythrophagocytosis in the liver and of hemosiderosis in the spleen. A survey of the occurrence of these processes in a number of cases examined at necropsy, with an attempt to correlate the splenic and hepatic changes, is also included. A similar analysis of the reported cases in the literature with complete necropsy data has also been attempted.

MATERIAL

From 4,094 postmortem examinations at Charity Hospital of Louisiana from January 1, 1939, to January 1, 1942, 12 cases of sickle cell disease were available for study. Cases with incomplete data were excluded. Data pertaining to the age, sex, size of the spleen and liver, and the degree of erythrophagocytosis and hemosiderosis were tabulated. In addition to the routine hematoxylin and eosin staining, the presence of hemosiderin was tested by the Turnbull-blue reaction.⁹

Since the anemia was not established in all cases, the term "sickle cell disease" will be used, rather than "sickle cell anemia."

REPORT OF ILLUSTRATIVE CASE

A colored male, 14 years of age (see case no. 3, Table II), was admitted with cough, and edema of the face. He gave a history of having had heart trouble for 1 year. Three years prior to admission he had had rheumatic fever.

* Received for publication, June 1, 1942.

Examination revealed a poorly nourished, but fairly well developed patient with a temperature of 99.6° F.; pulse, 110; respiration, 26; blood pressure, 116/40. The heart was markedly enlarged. A blowing systolic apical murmur was heard. The liver was markedly enlarged. On the anterior aspect of the legs there were numerous small scars present.

Laboratory Findings. The blood examination was as follows: Hemoglobin, 5.8 gm. per 100 cc., 40% (Hellige); red blood cells, 1,780,000; white blood cells, 19,000; nucleated red cells, 60,000, and platelets, 500,000 per cmm.; hematocrit, 21.5 (Wintrobe); polymorphonuclears, 48 per cent; eosinophils, 4 per cent; monocytes, 9 per cent; lymphocytes, 25 per cent; promyelocytes, 2 per cent; myelocytes, 9 per cent, and metamyelocytes, 3 per cent. The wet preparation showed 90 per cent of the red blood cells to be sickle-shaped. The fragility of the red cells was normal. A sternal puncture revealed a hyperplastic, pronormoblastic marrow. Clotting time and coagulation time (Lee-White) were within normal limits. The urine showed no deviation from the normal. The icterus index was 66; the van den Bergh test showed 7.6 mg. of bilirubin per 100 cc. (direct method). Four days later the van den Bergh test gave 25 mg. of bilirubin per 100 cc. (direct method). The Kline flocculation test was negative. On several occasions the sputum was negative for acid-fast bacilli. Roentgenograms of the chest showed far advanced bilateral pulmonary tuberculosis and cardiac enlargement. The electrocardiograph showed evidence of myocardial disease typical of rheumatic mitral stenosis.

Clinical Course. Patient became progressively more anemic and developed jaundice, dyspnea, and pitting edema of both legs. His temperature became of septic type, and he expired 77 days after admission to the hospital.

Necropsy Findings

There was marked edema of the face and lower extremities. Both legs presented numerous scars on the anterior aspects. The heart weighed 500 gm. and showed marked right ventricular hypertrophy and mitral stenosis. The lungs presented a far advanced exudative tuberculosis with numerous cavities. Enlarged caseous hilar nodes were present. The spleen was small, firm, purplish in color and weighed 24 gm. Its cut surfaces were fibrous. The liver weighed 3600 gm., and the cut surfaces revealed diffusely scattered grayish yellow areas measuring 1 mm. in diameter. The kidneys were markedly enlarged, weighing together 720 gm. The sternum, thoracic vertebrae and the middle portion of the femur presented red, active marrow.

Histologic Examination

A large proportion of the red blood cells were sickle-shaped in all organs. The sinusoids of the liver were markedly distended; the Kupffer cells were enlarged and their cytoplasm contained numerous sickle-shaped red blood cells (Fig. 4). The liver cells were compressed and contained finely granular cytoplasm, with well stained nuclei. Occasional small areas of extramedullary myelopoiesis were seen. Some bile capillaries contained bile plugs. The spleen showed a marked increase in connective tissue. Around the blood vessels large amounts of dark brownish green pigment were deposited, which varied in intensity. Also aggregations of dark blue incrustations were evident. The

lymph follicles were small and without germinal centers. Some sinusoids were distended with sickle-shaped red blood cells. The walls of the sinusoids were thickened and the sinus endothelial cells were conspicuous, occasionally containing phagocytized red blood cells. In addition to the tuberculous lesions, the lungs showed large alveolar phagocytes containing sickle-shaped red blood cells. The kidneys showed hyperemia, interstitial edema and small areas of erythropoiesis. Active pronormoblastic erythropoiesis was seen in the bone marrow. The mesenteric lymph nodes presented small follicles and markedly distended sinusoids, with active erythrophagocytosis by sinusoidal cells. The Turnbull-blue stain was strongly positive in the spleen and slightly positive in the liver, kidneys, bone marrow and mesenteric lymph nodes.

Summary of Case

In the case presented the spleen was small and fibrous with calcium and iron incrustations. The liver was greatly enlarged (Fig. 6) and exhibited a marked degree of erythrophagocytosis. The various portions of the reticulo-endothelial system appeared to be in quite different functional stages. The splenic reticulo-endothelium appeared to be inactive while the Kupffer cells exhibited marked hyperactivity.

OBSERVATIONS, INCLUDING ADDITIONAL CASES

The 12 cases were divided into two groups according to the size of the spleen. The first group (Table I) included 7 cases in which the spleen was of normal weight or enlarged (average 286.4 gm.). The

TABLE I
Cases of Sickle Cell Disease with Spleens of Normal Weight, or Larger

Case no.	Age	Sex	Spleen gm.	Liver gm.	Liver-spleen ratio	Erythro-phagocytosis		Hemosiderosis		Complicating disease
						Spleen	Liver	Spleen	Liver	
1. W. S.	28	M	140	1535	10.96	—	+	—	—	Brain abscess
2. E. H.	47	M	160	1720	10.75	—	—	+	+	Acute thyro-toxicosis
3. L. M.	40	F	225	2400	9.60	+	—	+	—	Typhoid fever
4. M. S.	6	M	70	640	9.14	—	+	+	+	Wilms' tumor
5. A. L.	49	M	150	2200	14.66	—	++	++	—	Type 23 pneumococcus meningitis
6. J. W.	54	M	190	1790	9.42	+	++	—	—	Hypertension
7. Z. K.	17	M	1070	1650	1.54	+	++	+	—	Abdominal crisis
Average	34.4		286.4	1705						

livers of this group were of normal weight or moderately enlarged (average 1705 gm.). The average age of the patients in this group was 34.4 years, the ages ranging from 6 to 54 years.

The second group (Table II) consisted of 5 cases in which the spleen was small (average 19.7 gm.). The livers varied from a normal size to markedly enlarged (average 2106 gm.). The average age was 19 years, the ages ranging from 14 to 39 years.

Because of the wide age range in both groups the hepatic-splenic ratio was computed according to the method given by Ahronheim,¹⁰

TABLE II
Cases of Sick Cell Disease with Small Spleens

Case no.	Age	Sex	Spleen gm.	Liver gm.	Liver-spleen ratio	Erythro-phagocytosis		Hemosiderosis		Complicating disease
						Spleen	Liver	Spleen	Liver	
1. E. L.	22	M	5.4	1950	361.1	—	+++	—	+	Abdominal crisis
2. E. R.	39	F	7.0	1250	178.6	—	—	+++	—	
3. C. O. H.	14	M	24.0	3600	150.0	++	++	+++	+	Tuberculosis
4. W. M. L.	18	F	50.0	3080	61.6	—	+	+++	—	Postpartum thrombophlebitis
5. F. W.	2	F	12.0	650	54.2	+	—	++	—	Rheumatic fever
Average	19.0		19.68	2106						

who found that the normal ratio between hepatic and splenic weight is 9.3:1. The ratios for the cases tabulated in Table I were close to or slightly above this value, with two exceptions—cases nos. 5 and 7. Case no. 4, a boy of 6 years, showed absolute values above the normal for his age,¹¹ but the hepatic-splenic ratio was within normal limits. The cases tabulated in the second group showed very high values for the ratio, indicating a marked diminution of the spleen and enlargement of the liver in all instances.

Histopathology of the Liver

The livers were enlarged, reddish brown and firm. Histologic study revealed marked congestion, the sinusoids being distended and filled with sickle-shaped red blood cells. The Kupffer cells were swollen and exhibited varying degrees of erythrophagocytosis. This was most marked in case no. 1, Table II (Fig. 4). The liver cords were regularly arranged and the parenchymal cells were compressed or slightly swollen and granular. Infrequently lipoidosis in varying degrees was seen.

Histopathology of the Enlarged and Normal-Sized Spleens

The spleens of the first group revealed a marked congestion of the sinusoids and venous sinuses, which were filled with sickle-shaped red blood cells. The lining cells of the sinusoids formed thin, irregular, netlike structures. The central arteries were also markedly dilated and the follicles were without germinal centers. Often there were pools of extravasated blood around the follicles. This was most marked in case no. 7, Table I, in which the spleen weighed 1070 gm. (Fig 5). Varying amounts of iron-containing pigment were found. The reticulum showed in places a somewhat thicker fibrillar network, upon which hemosiderin was deposited in the form of myceliumlike threads (Fig. 1). These changes were often confined to the vicinity of the small blood vessels. Around the periadventitial tissue the reticulum coalesced to form a thick hyaline network, and here the Turnbull-blue stain revealed thick rods of deposited iron (Fig. 2). In irregular areas calcium deposits were also present and small hemorrhages and old infarcts of small size were often seen.

Histopathology of the Fibrotic Spleens

There was an increase of hyaline connective tissue and widening of the trabeculae in the fibrotic spleens. In this connective tissue, irregular areas of dark bluish green or brownish clumps, often arranged in parallel rods resembling bamboo sticks, were formed. In other instances, the deposits formed structureless bluish brown masses (Fig. 3). The walls of the sinusoids were thickened, and their endothelial lining cells were flattened. Distorted red blood cells filled the sinusoids. Erythrophagocytosis by the sinus endothelial cells was minimal. The follicles were inconspicuous and without germinal centers. The iron stain was strongly positive in all but one instance (case no. 1, Table II), in which the spleen was very small and in which the parenchyma was replaced by fibrous connective tissue.

REVIEW OF CASES FROM THE LITERATURE

A large number of cases of sickle cell disease have been reported but complete necropsy data are available in only a limited number. In reviewing the literature, only 13 cases were found in which there were included data pertaining to the age, sex, size of the spleen and liver, and the degree of erythrophagocytosis and hemosiderosis. These cases have been grouped according to the size of the spleens.

The first group consisted of 5 cases with normal or enlarged spleens (Table III). No average can be given since only one adult was included in this group, but the hepatic-splenic weight ratio clearly

indicates splenomegaly. Case no. 1, a colored girl, 4 years of age, was included because the weights of her spleen and liver were within normal limits.¹¹ The remainder of the cases showed a definite splenomegaly.

The second group consisted of 8 cases with siderofibrotic spleens and correspondingly high values for the hepatic-splenic ratios (Table IV). The age range in this group was from 6 to 38 years, the average age being 20.5 years. The spleens again were markedly atrophic

TABLE III

Collected Cases of Sickle Cell Disease with Spleens of Normal Weight, or Larger

Author and year	Age	Sex	Spleen gm.	Liver gm.	Liver-spleen ratio	Erythro-phagocytosis		Hemosiderosis		Complicating disease
						Spleen	Liver	Spleen	Liver	
Wollstein and Kreidel, ⁵ 1928	4	F	30.0	460	15.3	—	++	++	+	
Wollstein and Kreidel, ⁵ 1928	3	M	210.0	460	2.2	—	++	+	+	
Wollstein and Kreidel, ⁵ 1928	3	M	182.0	430	2.4	—	++	+	+	
Lash, ¹⁴ 1934	21	F	960.0	2420	2.5	—	—	—	—	Cervical cesarean section
Ryerson and Terplan, ¹⁵ 1935	4	F	425.0	500	1.2	++	++	++	—	

(average 15.4 gm.). The livers were of normal size or enlarged (average 1834 gm.), as indicated by the uniformly high values for the liver-spleen ratios.

COMMENT

This survey indicates that varying degrees of erythrophagocytosis were present in sickle cell disease. The Kupffer cells of the liver most frequently exhibited engulfed red blood cells, while the splenic reticulum cells only occasionally showed signs of erythrophagocytosis. The changes in the spleen, however, were more varied in nature and more severe than those of the liver. An abnormal distention of the perfollicular sinusoids, hemorrhages and hemosiderosis constituted the different stages of the splenic lesion, producing the siderofibrotic spleen.

In the literature markedly enlarged spleens were reported chiefly in young children (Table III). There was only one adult in this group, a female, 21 years old. Cases with normal spleens, or with

spleens but slightly smaller than normal, were included in Table I. These probably are the less severe forms of sickle cell disease, as death in all instances was due to complicating conditions.

The cases tabulated in Tables II and IV are the severe forms of sickle cell disease with siderofibrotic spleens. Assembled respectively from the records of Charity Hospital of Louisiana and from the reported cases in the literature, these two groups show remarkable similarities.

TABLE IV
Collected Cases of Sickle Cell Disease with Small Spleens

Author and year	Age	Sex	Spleen gm.	Liver gm	Liver-spleen ratio	Erythro-phagocytosis		Hemosiderosis		Complicating disease
						Spleen	Liver	Spleen	Liver	
Graham, ¹⁶ 1924	30	F	28.0	2567	92.0	—	+	+++	+	Streptococcus septicemia
Sydenstricker, ¹² 1924	6	M	7.9	690	87.0	—	—	+	—	
Jaffé, ¹⁷ 1927	8	M	10.0	900	90.0	—	++	++	—	Tuberculosis
Jaffé, ¹⁷ 1927	6	F	10.0	1300	130.0	—	++	++	+	
Ching and Diggs, ¹⁸ 1933	18	F	10.5	2210	210.0	—	+++	—	—	
Yater and Hansmann, ¹⁹ 1936	38	F	7.0	1715	245.0	—	—	+++	+	Abdominal crisis
Yater and Hansmann, ¹⁹ 1936	25	F	35.0	2090	59.7	—	—	+++	++	Abdominal crisis
Bauer, ²⁰ 1940	33	F	15.0	3200	213.0	+	+	++	—	
Average	20.5		15.4	1834						

From the available clinical data it is difficult to establish the duration of active sickle cell disease. Diggs,⁸ who studied thoroughly the changes in the spleen in sickle cell anemia, concluded that there is no direct correlation between the size of the spleen and the duration of the disease as indicated by the age of the patient. A large spleen was usually found in the early phases of the disease, while a small atrophic spleen was characteristic of the later phases. This is, however, not without exception. Therefore, the duration of the disease could be determined more accurately by the histologic changes present.

Cases included in Table I were characterized by normal or enlarged spleens, with histopathologic changes suggestive of shorter duration, and cases in Table II revealed siderofibrotic spleens, indicating longer duration. It is difficult to correlate these changes with the duration of the disease when one considers that the average age of the first group was 34.4 years and of the second, 19.0 years. There exist both active and latent varieties of sickle cell disease,¹² differing in degree rather than in kind. The first group probably represented the latent cases, and the second group the active cases. However, the pathologic changes differed only in degree. The enlarged spleens and the normal-sized spleens presented similar pathologic changes, differing again only in degree. In the active cases the liver-spleen ratios were much lower (Table III) or much higher (Tables II and IV) than normal, while in the latent form (Table I), they were nearly normal.

No direct correlation existed between erythrophagocytosis and complicating infectious disease¹³ (Tables I to IV).

SUMMARY

An example of sickle cell disease is reported in which there was a very marked degree of erythrophagocytosis.

Twelve cases of sickle cell disease were studied from 4,094 autopsies. These were divided into two groups: those with large and normal-sized spleens and normal or slightly enlarged livers; and those with siderofibrotic spleens and enlarged livers. These correlations were definitely demonstrated by the liver-spleen ratios.

Thirteen cases with complete necropsy data were collected from the literature and also tabulated according to the weight of their spleens. Definite splenomegaly was present in 4 cases. There was a normal-sized spleen in one case, and eight cases showed marked siderofibrotic changes with high values for the liver-spleen ratio.

The Kupffer cells were the most actively participating parts of the reticulo-endothelium in erythrophagocytosis. This process is not dependent upon coexisting infection.

The splenic changes may serve as criteria for the degree of activity but not for the duration of the sickle cell disease. The latent cases also present definite changes in the spleen and liver similar to those seen in active cases. The spleen may be enlarged, normal, or markedly fibrosed in sickle cell disease.

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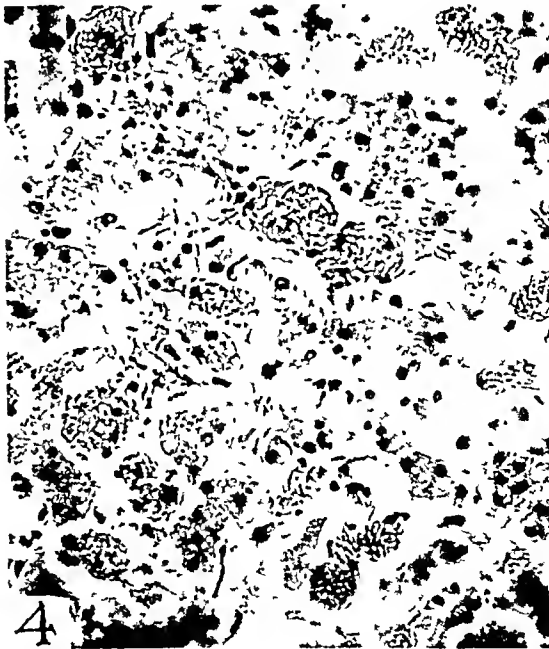
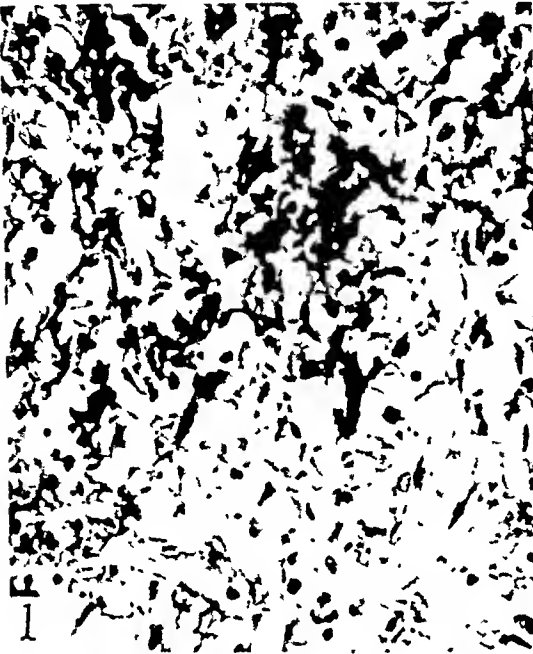
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 27

- FIG. 1. (Case no. 4, Table II.) Spleen: marked distention of the sinusoids. Hemosiderin has been deposited in myceliumlike fashion. Turnbull-blue stain. $\times 300$.
- FIG. 2. (Case no 4, Table II.) Spleen: rodlike deposits of hemosiderin. Turnbull-blue stain. $\times 335$.
- FIG. 3. Case no. 1, Table II.) Spleen: a typical siderofibrotic area with marked hemosiderin and calcium incrustation. Turnbull-blue stain. $\times 45$.
- FIG. 4. (Case no. 1, Table II.) Liver: the Kupffer cells show a marked degree of erythrophagocytosis. Hematoxylin and eosin stain. $\times 335$.



Stasney

Erythrophagocytosis in Sickle Cell Disease

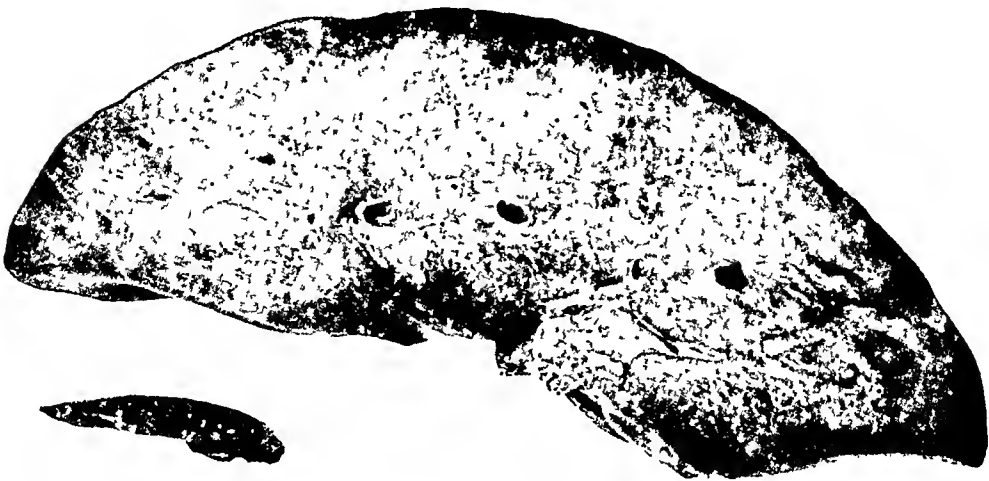
PLATE 28

FIG. 5. (Case no. 7, Table I.) Spleen: markedly distended sinusoids. Sickie-shaped red blood cells are found in the pool around the lymph follicle. Hematoxylin and eosin stain. $\times 100$.

FIG. 6. (Case no. 1, Table II.) Spleen and liver: marked disproportion in size.



5



6

Erythrophagocytosis in Sickle Cell Disease

Stasney



HISTOLOGICAL CHANGES PRECEDING SPONTANEOUS LYMPHATIC LEUKEMIA IN MICE *

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According to its characteristic manifestations, leukemia is a generalized, or systemic, disease. However, the histogenesis of leukemia has been debated for many years and numerous theories have been presented. As Richter¹ pointed out in a recent review: "The various theories may be roughly grouped in two categories: according to one, the leukemic cells reach their unusual situations by metastases from their normal sites of origin. . . . ; according to the other, the cells are formed *in situ* where they are found." The observations herewith presented suggest that part of each of these categories may be correct, namely, that leukemia may develop by metastases following origin in abnormal sites.

Theories have been given free rein because material for the critical study of this question is rare. The absence of early clinical signs of leukemia has limited histological studies to relatively late stages, after the initial histogenesis has become confused, if not entirely hidden, by extensive proliferation.

Most of the discussions on the histogenesis of leukemia have been based on the examination of human material. Some of the more recent reviews and reports on leukemia and allied diseases have been presented, with consideration of current interpretations, by Richter,¹ Watson,² Klemperer,³ Stasney and Downey⁴ and Ehrlich and Gerber.⁵ In the field of experimental leukemia the subject has been touched on only rarely. Hill⁶ described various types of lymphoid hyperplasia in mice and concluded that leukemic cells were derived from the reticulum and that the first infiltrations occurred "in that portion of the mesentery binding the pancreas and the gut in region of the pylorus." Furth and Kahn,⁷ from their work on transplantable leukemias, suggested that a malignant change in a single lymphocyte, rather than a systemic change, might be responsible for the origin of spontaneous leukemia. Barnes and Sisman⁸ believed that their studies on malignant and nonmalignant myelosis did not answer the question concerning the origin of leukemic cells.

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SOURCE OF MATERIALS STUDIED

The genetic aspects of the high incidence of spontaneous leukemia in mice of strain C58 have been discussed by MacDowell and Richter⁹ and MacDowell.¹⁰ Victor and Potter¹¹ have reported on the metabolic differences occurring in preleukemic mice of this same strain as compared with mice of the same age group from nonleukemic strains. Since 90 per cent of the mice of strain C58 that live longer than 6 months die with some form of leukemia, it was assumed that a fair proportion of these mice of leukemic age and still clinically normal would show, histologically, the significant early stages. Therefore 137 mice between the ages of 176 and 382 days, but clinically nonleukemic, were killed and the tissues from all organs saved. Most of the animals were obtained from retired mating pens and both sexes were represented, with females predominating (107 females to 30 males). The material was collected in 1933 and 1934, with additions in 1937 and 1938. The tissues from these preleukemic C58 mice were compared with tissues from a group of animals within the same age range from the nonleukemic strains, Storrs-Little and Bagg-albino, as well as with tissues from young C58 mice 6 to 8 weeks old.

All of the C58 and other strains of mice used in the present and preceding studies were bred and raised in the same colony under the same conditions of housing, diet and care.

A second source of material was a series of monthly biopsies performed by one of us (J. V.) for the study of the metabolic history of lymphatic tissues in individual mice during the period preceding leukemia. As planned, ten lymph nodes were to be removed singly from each of 24 mice of strain C58 at monthly intervals, beginning with animals 2 months old and omitting a biopsy at 3 months. At the end of this period, or when death occurred from any cause, all of the remaining tissues were to be examined microscopically. The method of biopsy, of course, provided only a small amount of isolated tissue at each period, the condition of which could not be compared with the animal's remaining tissues at the same time. The method, however, furnished helpful information on the probable sequence of events during the histogenesis of leukemia.

A third set of observations was made on tissues from 40 C58 and 10 Storrs-Little mice killed at intervals in connection with another experiment.

Tissues were fixed in Helly's or Zenker's fluids, with other methods being used for special purposes. Hematoxylin and eosin or hematoxylin and eosin with azur II were used for routine staining, but

other stains were used at times to accentuate certain histological or cytological characters. Flemming's fluid and triple staining were found useful for nuclear detail in small pieces of tissue.

OBSERVATIONS

In 59 of the 137 C58 mice examined *in toto*, reticulum cell hyperplasia was observed that had not been seen in young C58 mice or old mice of nonleukemic strains. In general the hyperplasia was confined to the reticulum of lymph nodes or to the perivascular regions of the livers. The spleen seemed to be involved in only 3 animals. The distribution of the hyperplasia was limited and in no case was there a generalized systemic reaction. When it occurred in lymph nodes, only one or two nodes were usually affected. In livers the lesions were not widely spread but were confined to a few foci. The hyperplasia occurred in both liver and lymph nodes in some cases, while in others only one or the other tissue was the seat of change. When a single node of a group was affected, *e.g.*, one of the cervical group, other nodes in the same group were usually unchanged. By designating a single node or liver tissue as a region, the 59 animals showed the following distribution: 1 region in 18 cases, 2 in 22 cases, 3 in 11 cases, 4 in 7 cases, and 5 in 1 case.

The 59 animals showing hyperplasia were of various ages and the changes appeared in both sexes (47 females and 12 males). These animals represented 44 per cent of the total females and 40 per cent of the total males examined. A description of the reticulum changes as they appeared in lymphoid and hepatic tissues follows.

Lymph Nodes

The earliest signs of reticulum hyperplasia in lymph nodes seemed to be confined to the medullary regions. In some cases only a small area was involved, while in others the reticulum cells displaced the remaining elements of the node to a great extent. Depletion of the free cells of the nodes, leaving the normal reticulum more apparent, could not account for the microscopical picture because of definite evidence of displacement of normal elements by the hyperplastic growth of the reticulum cells. It has not been possible to associate this reaction in C58 mice with any condition other than a susceptibility to spontaneous leukemia. Further evidence that this latter statement is valid was presented by MacDowell and Richter,⁹ who showed that affections other than leukemia were rare in the 637 C58 mice examined by them at autopsy.

The reticulum cells were judged to be more compactly arranged than in normal regions, and there was no evidence that the hyperplasia originated in the follicles of the cortex. In extreme cases the normal architecture of a node was obliterated by the hyperplastic reticulum. This phenomenon was associated with a gross enlargement of the involved node in 9 cases.

There was no indication that the hyperplasia occurred in nodes of one region more often than in those of another. Similar histological pictures were seen in several instances in two nodes which were widely separated in the host's body. "Diffuse follicular hypertrophy" was not observed in any case during the preleukemic stages in C58 mice, although this condition has been said to be associated with the onset of leukemia.²

Liver

Simultaneously with, or independently of, changes in lymph nodes, reticulum hyperplasia was observed in the liver. The earliest stages of this proliferation appeared as small nodules of syncytial reticulum cells apparently originating from the perivascular reticulum. The extent of the proliferation varied from site to site and from animal to animal, but in no case was the hyperplasia general for all perivascular regions within a liver. Reticular fibers could be demonstrated in the lesions. In advanced stages, limits of the individual reticulum cells were more easily identified and various degrees of differentiation of cells of the lymphatic series were associated with the area of reticulum hyperplasia. Examples of this type of lesion from 4 different animals are shown in Figures 1-4. In terminal cases the participation of the reticulum was marked by progressive proliferation of lymphoid cells.

Spleen

Reticulum hyperplasia occurred in only three spleens. In these cases the affected areas were small and nodular, in close proximity to trabeculae but not associated with malpighian follicles. Perhaps because of the normally confused and shifting cellular population, histological and cytological changes in splenic tissue must be more pronounced in order to become assuredly apparent. Most certainly the point of origin of a single free cell in the red pulp of the spleen is a matter of conjecture, due to the continually changing topographical relationship of the free cells. It has been possible to assign only a minor part to the spleen in the origin of the leukemic state in C58 mice.

Other Tissues

All lesions in the remaining tissues were infiltrative in character, with no evidence of local origin from fixed tissues. After proliferation of malignant free cells began at any site, transport and invasion became widespread and could account for most of the lesions found in terminal cases. It is impossible to determine from these studies on our material how long the preleukemic stages exist before the disease becomes clinically apparent, but it seems obvious that there would be considerable variation in this respect.

Response of Preleukemic Lesions to Carmine Injections

As a possible means of obtaining a measure of the functional state of the reticulum cells in the preleukemic lesions, a group of 11 animals of leukemic age were given injections of carmine. Of this group, 5 animals were found to have either lymph node or liver changes, or both, of the desired types. The amount of carmine injected was sufficient for general distribution throughout the animal, but in no case was there a concentration of the dye within the areas of reticulum hyperplasia described above. However, the more clearly differentiated cells, such as the Kupffer cells of the liver, known to be phagocytic, were loaded with particles of dye and were often in close proximity to the hyperplastic areas. We interpret this result as showing that the stimulated reticulum in the preleukemic lesions of C58 mice did not have histiocytic tendencies under the existing conditions. This evidence is considered of importance because of the general acceptance of the lymphopoietic potency of nonstoring cells of the reticulum.

Biopsy Experiments

Instead of making available all the tissues from one animal at a given moment, the biopsy experiments gave samples of lymphatic tissue from the same animal at regular intervals. Such successive samples revealed directly the relationship between reticulum hyperplasia and lymphopoiesis on the one hand and leukemia on the other.

The results of histological analysis of the individual nodes at biopsy and of all tissues at autopsy are given in Table I. The animals are grouped according to the terminal diagnosis; those which died early either without leukemia or undiagnosable on account of postmortem change are given, but do not contribute directly to answering the question at hand.

The significant point is that the 10 cases of leukemia at autopsy were preceded without exception by reticulum hyperplasia and

TABLE I
Histological Condition of Individual Lymph Nodes at Biopsy

Animal no.		Diagnosis based on all tissues at autopsy																											
		Leukemic												Not leukemic										Early death without diagnosable leukemia					
Lymph node	Age mos.	6	8	10	13	14	15	17	18	19	20	3	4	5	12	16	21	22	22	23	24	1	2	7	9	11	23	24	
1st	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2nd	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3rd	5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4th	6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5th	7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6th	8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7th	9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8th	10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9th	11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10th	12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N = Normal
h = reticulum hyperplasia
d = lymphopoiesis
+ = marked
L = histological leukemia
- = insufficient tissue obtained
* = post mortem changes precluded diagnosis

lymphopoiesis. Five of these mice died with leukemia before the end of the experiment and 5 showed leukemia histologically when killed. That similar reticulum hyperplasia occurred in 6 cases without leukemia at autopsy is not significant, since in each of these cases the outcome was concealed by the arbitrary killing after the tenth node was removed.

Animal no. 20 gave the most orderly sequence of stages approaching leukemia; photomicrographs of sections from these tissues are shown in Figures 5-7. However, the frequent failure of successive nodes from the same animal to show a regular progression (7 cases) is evidence that the initial stages do not occur uniformly throughout the lymphoid system. In 2 cases (animals nos. 6 and 18) the tenth node did not show leukemia, but other tissues did. With such irregularity in the distribution of the early manifestations, it is entirely possible that the removal of an especially active node may have altered the course of events.

These observations on tissues from the same animal covering a period of 10 months confirm the conclusion that reticulum hyperplasia precedes the spontaneous production of leukemia cells.

Miscellaneous Series

In connection with another experiment further evidence was obtained showing that reticular changes are typical of strain C58 mice after the age for spontaneous leukemia is reached. From a group of 40 C58 and 10 Storrs-Little (a nonleukemic strain) mice of the same age, individuals were killed at intervals and examined histologically. Of the 24 C58 mice killed before 6 months of age none showed reticular hyperplasia; of the 16 killed at 6 months, 7 showed reticular hyperplasia identical with that reported above in either lymph nodes or liver. The Storrs-Little animals showed no reticular hyperplasia at any time (7 killed before and 3 killed at 6 months).

DISCUSSION

To demonstrate conclusively the formation of one type of cell from another type by present histological methods it is necessary to have a picture of progressive zonal development, as for example in the formation of spermatozoa. With the lack of delimited areas of progressive differentiation it is usual to construct a closely knit series dependent on overlapping morphological criteria. This latter method has been the one used in the studies on blood cell formation, and the subjective reactions involved may perhaps explain in part the long-drawn-out controversy on the subject. However, there is almost unan-

imous agreement among present-day investigators that lymphocytes may be derived from undifferentiated, nonstoring multipotent cells of the reticulum, a topic recently reviewed by Bloom.¹²

In the absence of a generalized hyperplasia of lymphoid tissues during the preleukemic period in C58 mice, the localized reticulum hyperplasia (absent in nonleukemic strains) and associated lymphopoiesis become important in considering the source of leukemic cells. In concluding that the immature lymphocytes which appear in the areas of reticulum hyperplasia originated from the reticulum cells, the morphological method was used and the possibilities of subjective error are admitted. However, three observations can hardly be questioned: the failure of immature cells to leave lymph nodes during the preleukemic period, the inactive state of germinal centers in lymph nodes, and the presence of cell intergradations from reticulum cell to lymphocyte in the regions designated.

Accepting the reticulum lesions described here as sites for primary leukemic cell production of a focal nature, the widespread terminal infiltrations may readily be explained. After the formation of lymphocytes with a leukemic constitution, the motility of the lymphocyte and the ease with which it is transported within the lymphatics and the blood stream offer acceptable explanations for the formation of metastases which could account for the systemic infiltrations in terminal cases. Further support for belief in this metastatic type of lesion comes from the studies on the characteristics of leukemic cells during transfer.

Transplantation of experimental leukemias has produced strong evidence that each leukemic cell population is autonomous and that the final distribution of lesions depends on the site of entry, the intrinsic characteristics of the particular transplanted leukemic cells studied and the interaction between the host and the cells injected.¹³⁻¹⁵ Assuming that populations of leukemic cells arising spontaneously have similar intrinsic characters which determine the tissues which they will infiltrate, the point of origin of cells studied in terminal lesions of a spontaneous case becomes a matter of conjecture. Since Hill's⁶ statements concerning the histogenesis of leukemia and allied diseases in her strain of mice were based upon observations on terminal cases, they can have little significance in interpreting early stages.

As pointed out before, in the early stages of spontaneous leukemia, when the number of lesions is small, two types of lesions are regularly found: one with reticular activity associated with the production of lymphocytes, and the other with characteristics of the metastatic type

found in transplanted leukemias. In the terminal phase evidence of participation by the reticulum disappears.

Although we have found no evidence of participation by the normally cytopoietic reticulum in germinal centers of lymph nodes in the development of spontaneous leukemia in C58 mice, Ehrlich and Gerber⁵ found such evidence in the development of lymphocytic neoplasia in human material. Their evidence for progression from a lesion of reticular type to a lymphocytic type is clear. From a theoretical standpoint it is possible for any lymphopoietic tissue to respond to the unknown stimuli which result in the production of leukemia and allied diseases. The important part that the genetic constitution plays in the determination of neoplasia has been clearly demonstrated. The consistent preleukemic changes in C58 mice may be due to the genetic constitution of the strain, and with the same stimulant against other genetic backgrounds a different, but perhaps just as consistent, picture might be obtained.

The multipotency of the reticulum cell precludes the possibility of identifying leukemic progression by the mere recognition of reticulum hyperplasia without observations of later stages. Klemperer³ has warned of such misinterpretation, and has shown the importance of the reticulum in diseases of the hemopoietic system, as have Stasney and Downey⁴ and others.

A few authors have questioned the conception of leukemia as a disease of systemic origin. In a review, Watson² called attention to "the unquestioned existence of leukemia in which the anatomic lesions are well localized, with minimal or no changes elsewhere." This observation points toward a restricted origin of primary malignant change. Furth and Kahn,⁷ having concluded that leukemia in mice may be transmitted by a single cell, suggested that a malignant change in a single lymphocyte may be the origin of spontaneous leukemia. This hypothesis would eliminate the participation of fixed lymphopoietic tissues in the malignant change but would most certainly localize the origin of the disease. In connection with a discussion of the origin of leukemic cells, Furth¹⁶ also has pointed out that histological methods may lead to error in interpretation, and that the tissue culture technic has been of little value in the study of histogenesis of blood cells from fixed tissues. We agree that the final answer to the question of the spontaneous source of leukemic cells, and many normal cells for that matter, will have to wait until existing technics are perfected or new ones are discovered. However, at this time the great mass of evidence points to a direct relationship between the reticulum and the formation

of blood cells under normal and pathological conditions. The correlation between reticulum cell hyperplasia, abnormal lymphopoiesis and the onset of leukemia in C58 mice makes it necessary for us to conclude for the present that the spontaneous production of leukemic cells is the result of stimulation of fixed lymphopoietic tissues.

SUMMARY

We interpret our observations on tissues from preleukemic mice of strain C58 as showing that in this particular strain restricted areas of reticulum hyperplasia become the sites for primary malignant lymphocytopoiesis. These areas occur particularly in the medullary tissue of lymph nodes and in perivascular regions of the liver. Such areas have not been observed in nonleukemic strains. In mice of strain C58 hyperplasia of the germinal centers of lymphoid tissues was not observed in early stages of leukemia. Following the production of a population of free malignant lymphocytes, invasion accounts for the majority of the widespread lesions common to the terminal stages of leukemia. The concept of a widespread systemic origin of leukemia may have been due in part to the relatively late stage of the disease at which tissues have previously been examined.

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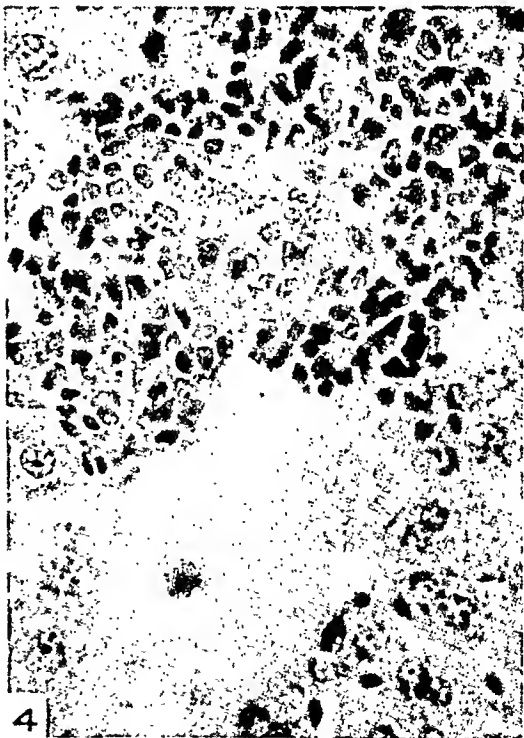
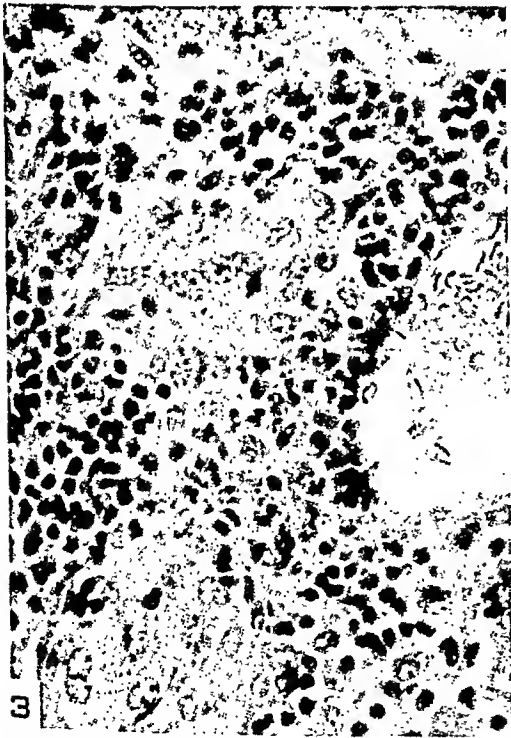
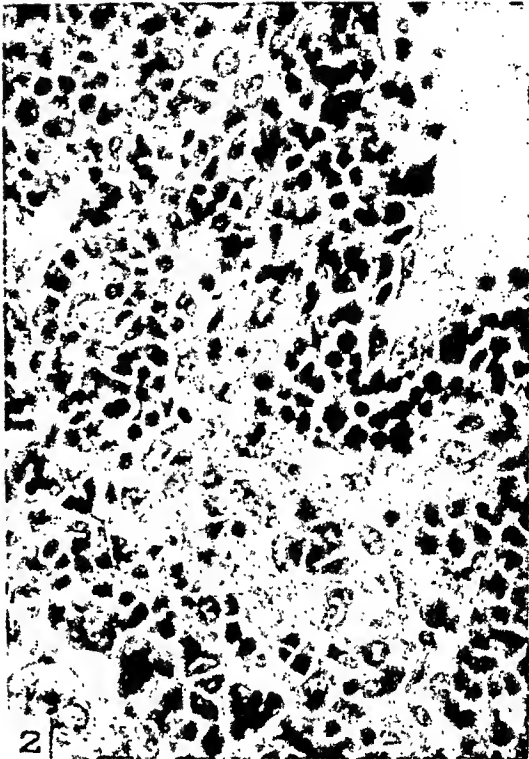
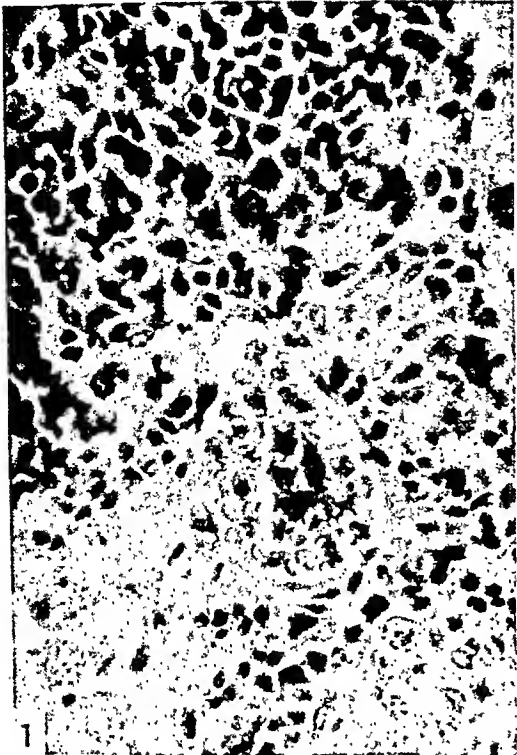
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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 29

FIGS. 1-4. Focal areas of periportal lymphopoiesis in the livers of four preleukemic C58 mice. $\times 460$.



Potter, Victor and Ward

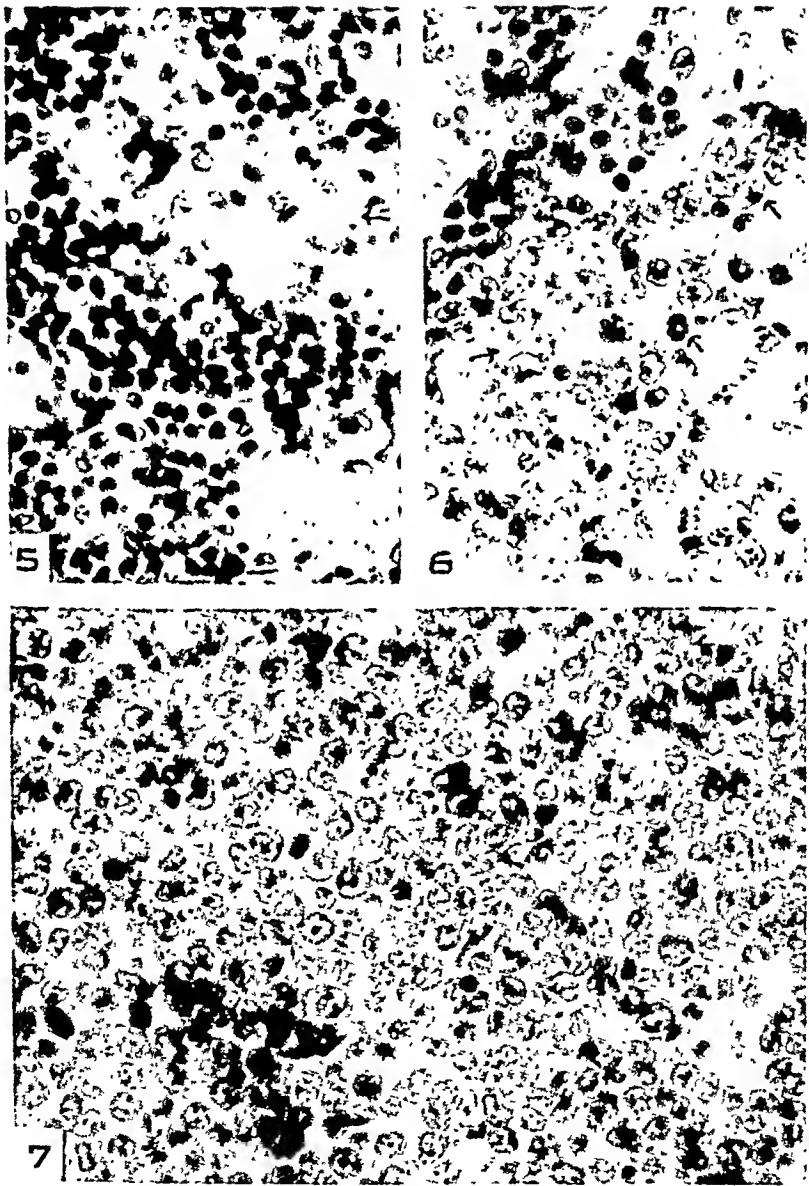
Changes Preceding Lymphatic Leukemia

PLATE 30

FIG. 5. Section of medullary tissue of lymph node removed from animal no. 20 (6 months old) at the fourth biopsy, showing reticulum cell hyperplasia and displacement of normal elements. $\times 460$.

FIG. 6. Section of medullary tissue of lymph node removed at the fifth biopsy from animal no. 20 (7 months old) showing reticulum cell hyperplasia and lymphocytopoiesis. $\times 460$.

FIG. 7. Leukemic lymph node removed from animal no. 20 (8 months old) at the sixth biopsy. $\times 460$.



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Changes Preceding Lymphatic Leukemia



IN VIVO NEUTRALIZATION OF PERTUSSIS TOXIN WITH PERTUSSIS ANTITOXIN *

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An interstitial mononuclear pneumonia similar to that occurring in pertussis has been produced in rabbits by intratracheal injection of cultures of the Bordet-Gengou bacillus,¹ by certain bacterial toxins,² and by viruses.³ Gallavan and Goodpasture⁴ showed that the Bordet-Gengou bacilli could be demonstrated only on the cilia of the bronchi and bronchioles and not in the lung tissue. Similarly, in chicken embryos they could demonstrate the Bordet-Gengou organisms only on the cilia of the bronchi, bronchioles and esophagus.

These findings suggested that the mononuclear reaction in the lungs could be the result of the action of some toxic substance produced by the bacilli in the bronchi and diffusing into the surrounding tissues. The test of this hypothesis was not possible, however, until a toxic substance capable of producing such a lesion could be isolated from pertussis organisms. Recently, Roberts and Ospeck⁵ reported the preparation of such a substance. This material is a heat-labile toxin obtained from cultures of the intermediate phases of the Bordet-Gengou bacillus. It will kill mice and produce necrosis in rabbit's skin. An antitoxin capable of neutralizing the action of this toxin was also described by these authors.

It was thought desirable (1) to determine if intratracheal injection of this toxin in rabbits could produce an interstitial mononuclear reaction similar to that occurring in pertussis, (2) to see if administration of antitoxin could neutralize or modify the lesion, and (3) to observe the effect of antitoxin administration on the lesions produced by injection of freshly isolated phase I Bordet-Gengou organisms. Regardless of whether pertussis in man results from infection by the Bordet-Gengou organisms alone, or represents the action of a combination of these organisms and a virus, it is generally recognized that the extensive damage to the lungs and bronchi is dependent upon the presence and multiplication of these bacteria. Hence, the demonstration of any inhibiting effect of antitoxin upon the mononuclear reaction in the lungs produced by these bacteria would be important in the control of this disease.

* Received for publication, June 10, 1942.

METHODS AND MATERIALS

Animals. Normal, adult, male, white rabbits weighing about 2 Kg. were used.

Toxin. The toxin employed was prepared from a phase II Bordet-Gengou bacillus and sent to us by Dr. M. E. Roberts of the Lederle Laboratories. The titration values of the toxin and antitoxin were determined by Dr. Roberts, who defined as a toxic unit (M. L. D.) the amount necessary to kill 50 per cent of mice 4 days after intravenous inoculation. This mouse unit is approximately 40 times the amount necessary to produce a skin lesion in the rabbit. Several batches of toxin with different titers were used, but in all cases 2 cc. amounts were injected directly into the trachea, after making an aseptic incision in the skin of the neck. Two cc. amounts were used as it was found that this amount of heat-inactivated toxin caused no significant pathologic changes in the lung.

Antitoxin. The antitoxin, made by the prolonged injection of the toxin into rabbits, also was supplied to us by Dr. Roberts. The unit of antitoxin as determined by Dr. Roberts was defined as that amount of antitoxin which would neutralize one M. L. D. of toxin. In our experiments the antitoxin was injected intravenously 24 hours before the intratracheal injection of toxin. The antitoxin, diluted 1 to 80, weakly agglutinated suspensions made of phase I, II, and III Bordet-Gengou organisms as well as a suspension of parapertussis bacilli.

Organisms. The Bordet-Gengou bacilli employed in these experiments were freshly isolated strains kindly supplied by Dr. J. G. M. Bullowa. The organisms were grown for 48 hours on a medium similar to that described by Bordet and Gengou⁶ except for the substitution of 20 per cent defibrinated human blood for horse blood. The growth on a large number of slants was washed off in sterile physiologic saline solution, pooled, rapidly frozen, lyophilized and sealed in a vacuum. This treatment was found not to damage the organisms. The approximate number of organisms injected was 100 billion (2 slants), although in some experiments 8 slants or about 400 billion organisms were used.

Necropsy. The animals dying within 4 days were necropsied shortly after death. The remainder were killed 96 hours after the toxin was injected, and necropsied immediately. The lungs were removed with the trachea, gently inflated with air and fixed in Zenker's solution. The sections were embedded in paraffin and stained with hematoxylin and eosin, and for bacteria according to MacCallum's method.⁷

EXPERIMENTAL

Toxin-Antitoxin Experiment

This experiment was designed to study the effect of intratracheal injection of the toxin on the rabbit's lung and the ability of the antitoxin to neutralize the toxin. Thirty-two rabbits were injected intratracheally with various amounts of toxin. Fifteen of these had received various doses of antitoxin intravenously 24 hours previously. The re-

TABLE I
Results Obtained in the Toxin-Antitoxin Experiment

No. of rabbit	Amount of antitoxin cc.	Strength of antitoxin units	Total no. of units	Amount of toxin cc.	Strength of toxin units	Total no. of units	Type of lesion
3	0	—	—	2	100	200	++++
4	0	—	—	2	100	200	++++
7	0	—	—	2	100	200	+++
8	0	—	—	2	100	200	++
22	0	—	—	2	100	200	++++
23	0	—	—	2	100	200	++++
24	0	—	—	2	100	200	Died 72 hr.
20	0	—	—	2	150	300	++++
21	0	—	—	2	150	300	Died 24 hr.
13	0	—	—	2	750	1,500	Died 72 hr.
14	0	—	—	2	750	1,500	Died 24 hr.
15	0	—	—	2	750	1,500	Died 24 hr.
16	0	—	—	2	750	1,500	Died 48 hr.
27	0	—	—	2	750	1,500	Died 24 hr.
28	0	—	—	2	750	1,500	Died 24 hr.
29	0	—	—	2	750	1,500	Died 48 hr.
30	0	—	—	2	750	1,500	Died 72 hr.
19	5	7,000 per cc.	35,000	2	100	200	++++
18	5	7,000 per cc.	35,000	2	163	326	+
17	5	7,000 per cc.	35,000	2	163	326	+
5	5	9,000 per cc.	45,000	2	100	200	++++
6	5	9,000 per cc.	45,000	2	100	200	+
25	7	7,000 per cc.	49,000	2	750	1,500	++
26	7	7,000 per cc.	49,000	2	750	1,500	++
31	7	7,000 per cc.	49,000	2	750	1,500	+
32	7	7,000 per cc.	49,000	2	750	1,500	++
1	6	9,000 per cc.	54,000	2	100	200	+
2	6	9,000 per cc.	54,000	2	100	200	+
9	15	7,000 per cc.	105,000	2	750	1,500	++++
10	15	7,000 per cc.	105,000	2	750	1,500	Died 24 hr.
11	15	7,000 per cc.	105,000	2	750	1,500	+
12	15	7,000 per cc.	105,000	2	750	1,500	+++

sults of these experiments are shown in Table I. Of 17 rabbits receiving no antitoxin, 10 died within 72 hours after injection and 5 of the remaining 7 had a reaction which we have described below as 4 plus. Those which died within 24 hours showed extensive edema. The others had necrosis and cellular proliferation similar to that which we have described as a 4 plus reaction. In these lungs there were large areas in which the alveoli and the alveolar walls were filled with large

mononuclear cells. These cells are thought to be both macrophages and alveolar epithelial lining cells in view of the work of Ross,⁸ who showed by supravital staining methods that the large mononuclear cells occurring in the alveoli of rabbits injected with staphylococcic toxin were both macrophages and epithelial lining cells. Around the blood vessels and bronchi were large numbers of lymphocytes. The blood vessels showed scattered areas of extensive endothelial hyperplasia. In other areas of the lung were large areas of necrosis (Fig. 1), some of which were infiltrated by numerous polymorphonuclear leukocytes. There were also areas in which hemorrhage and edema were prominent features. The bronchioles frequently showed some cellular infiltration and focal necrosis of the epithelium. One animal had a 3 plus reaction (Fig. 1) with changes similar to those described as 4 plus except for the absence of marked necrosis. The changes in the remaining rabbit were classified as a 2 plus reaction. The alveolar walls were thickened by large mononuclear cells. The perivascular lymphatics and the peribronchial tissues contained large numbers of lymphocytes. An occasional bronchiole showed a few round cells in the epithelial layer.

Of the 15 rabbits previously prepared with antitoxin, 7 showed a 1 plus reaction. A few macrophages were seen in both the interstitial tissue and the alveoli, and a moderate number of lymphocytes were found around the blood vessels and bronchi. Three rabbits had a 2 plus reaction, 4 had a 3 plus reaction, and 1 died with pulmonary edema within 24 hours after inoculation of the toxin.

From these results it is obvious that the toxin can produce results similar to those occurring in pertussis. The antitoxin, although it did not completely prevent the lesions, materially reduced them. The rabbits receiving large doses of antitoxin are discussed below.

Bordet-Gengou Organism-Antitoxin Experiments

Experiments were designed to determine whether the antitoxin would prevent or modify the lesions produced by the injection of freshly isolated Bordet-Gengou bacilli. Thirty-eight rabbits were used. The results and dosages are shown in Table II. Twenty rabbits received the antitoxin intravenously 24 hours before the organisms were injected. Of the 20 animals which received both organisms and antitoxin, 17 had only a 1 plus reaction and 3, a 2 plus reaction. Eighteen rabbits received the organisms alone: 2 of these died within 72 hours of injection, 2 had a 4 plus reaction, 9 had a 3 plus reaction, 4 had a 2 plus reaction and 1 had a 1 plus reaction.

There was no evidence of multiplication of the Bordet-Gengou

bacilli in the lungs. Hence it is thought that the lesions were the result of toxin formed by the disintegration of the organisms injected.

TABLE II
Results Obtained in the Bordet-Gengou-Antitoxin Experiment

No. of rabbit	Amount of antitoxin cc.	Strength of antitoxin units	Total no. of units	No. of organisms slants	Type of lesion
4	0	—	—	2	++
5	0	—	—	2	+++
6	0	—	—	2	+++
15	0	—	—	2	+++
16	0	—	—	2	+
13	0	—	—	4	+++
14	0	—	—	4	+++
19	0	—	—	4	++
20	0	—	—	4	+++
28	0	—	—	4	+++
29	0	—	—	4	+++
30	0	—	—	4	++++
33	0	—	—	4	++++
34	0	—	—	4	Died 72 hr.
35	0	—	—	4	Died 72 hr.
36	0	—	—	4	++
17	0	—	—	8	++
18	0	—	—	8	+++
12	6	6,000 per cc.	36,000	2	+
9	7	6,000 per cc.	42,000	4	+
10	7	6,000 per cc.	42,000	4	+
11	7	6,000 per cc.	42,000	2	+
8	8	6,000 per cc.	48,000	2	+
31	7	7,000 per cc.	49,000	4	+
32	7	7,000 per cc.	49,000	4	+
23	6	9,000 per cc.	—	4	+
1	10	6,000 per cc.	60,000	2	+
2	10	6,000 per cc.	60,000	2	+
3	10	6,000 per cc.	60,000	2	++
7	10	6,000 per cc.	60,000	2	+
21	7	9,000 per cc.	63,000	8	+
22	7	9,000 per cc.	63,000	8	++
24	7	9,000 per cc.	63,000	4	+
37	7	9,000 per cc.	63,000	4	+
38	7	9,000 per cc.	63,000	4	+
25	10	9,000 per cc.	90,000	4	+
26	10	9,000 per cc.	90,000	4	+
27	10	9,000 per cc.	90,000	4	++

From these experiments it is seen that the antitoxin neutralized much of the toxin.

The results of both experiments are summarized in Table III.

DISCUSSION

The pathogenesis of pertussis in man is an exceedingly complex problem. In the experiments cited here, as in our previous experiments,¹ the conditions cannot be compared to those found in the course of natural infection by the Bordet-Gengou organisms. In none of our previous experiments were we able to culture the organisms from the lungs of rabbits inoculated with large doses of Bordet-Gengou bacilli.

It was, therefore, our belief that the interstitial mononuclear reaction resulted from the action of some toxic substance released by the disintegration of the inoculated bacteria. If this were true, then it was not necessary to assume that a virus infection was present since it had been shown that bacterial toxins can produce a lesion identical to that caused by viruses.

The experiments presented in this paper further strengthen this thesis since it was shown that an interstitial mononuclear pneumonia

TABLE III
Summary of All Experimental Data

	Small doses					Large doses				
	Toxin (200-326 M. L. D.)					Toxin (1500 M. L. D.)				
	Died	++++	+++	++	+	Died	++++	+++	++	+
No antitoxin	2	5	1	1	—	8	—	—	—	—
Antitoxin 35,000-54,000 units	—	—	2	—	5	—	—	—	3	1
Antitoxin 105,000 units	—	—	—	—	—	1	—	2	—	1
	Bacteria (2 slants)					Bacteria (4-8 slants)				
No antitoxin	—	—	3	1	1	2	2	6	3	—
Antitoxin 36,000-49,000 units	—	—	—	—	3	—	—	—	—	4
Antitoxin 54,000-63,000 units	—	—	—	1	3	—	—	—	1	5
Antitoxin 90,000 units	—	—	—	—	—	—	—	—	1	2

can be produced by a cell-free, heat-labile substance isolated from Bordet-Gengou bacilli. The extent of this lesion also can be reduced by the previous injection of an antitoxin prepared from this material. Furthermore, the lesions caused by the injection of freshly isolated Bordet-Gengou organisms in phase I also are markedly reduced, if antitoxin is administered prior to the organisms.

The data summarized in Table III illustrate another interesting point, namely, that with large doses of antitoxin an occasional rabbit may give a more severe reaction when toxin is injected than if a smaller dose of antitoxin were given. Thus 1 rabbit died and 2 had a 3 plus reaction when injected with 1,500 M. L. D. of toxin after receiving 105,000 units of antitoxin. In contrast, of 4 rabbits receiving approximately half that amount of antitoxin, none gave more than a 2 plus reaction to the same dose of toxin. These findings suggest that the

lesions in the former group may have been caused in part by an allergic reaction due to passive sensitization to some other antigenic substance present in the toxin. No such reaction was observed in animals injected with Bordet-Gengou bacilli, presumably because the release of the material from the organisms was slower and the concentration less.

The results of these experiments cannot be applied directly to the problem of pertussis in man, since the organisms injected intratracheally in the rabbit do not multiply but rapidly disintegrate, thus releasing a large amount of toxin. In man, however, as Gallavan and Goodpasture⁴ have shown, the organisms multiply on the cilia of the bronchi and bronchioles. They disintegrate over a period of time, thus providing a steady supply of toxin to the lung over longer periods than in the rabbit. Hence, it is thought that the experiments in the rabbit were a more severe test of the neutralizing power of the antitoxin than would have been encountered had there been available an animal in which the organism could multiply. Of course, it will be necessary to test this antitoxin in the presence of the naturally occurring disease in man, but to us there seems to be every reason to expect that the lesions produced by the organisms will be inhibited by this antitoxin.

SUMMARY

Experiments are reported in which it is shown that a heat-labile toxin obtained from cultures of the intermediate phase of the Bordet-Gengou bacillus can produce an interstitial mononuclear reaction similar to that caused by the Bordet-Gengou bacillus.

It is also shown that an antitoxin prepared from this toxin, when injected prior to either the toxin or the Bordet-Gengou organisms, is capable of greatly modifying the extent of the lesion produced.

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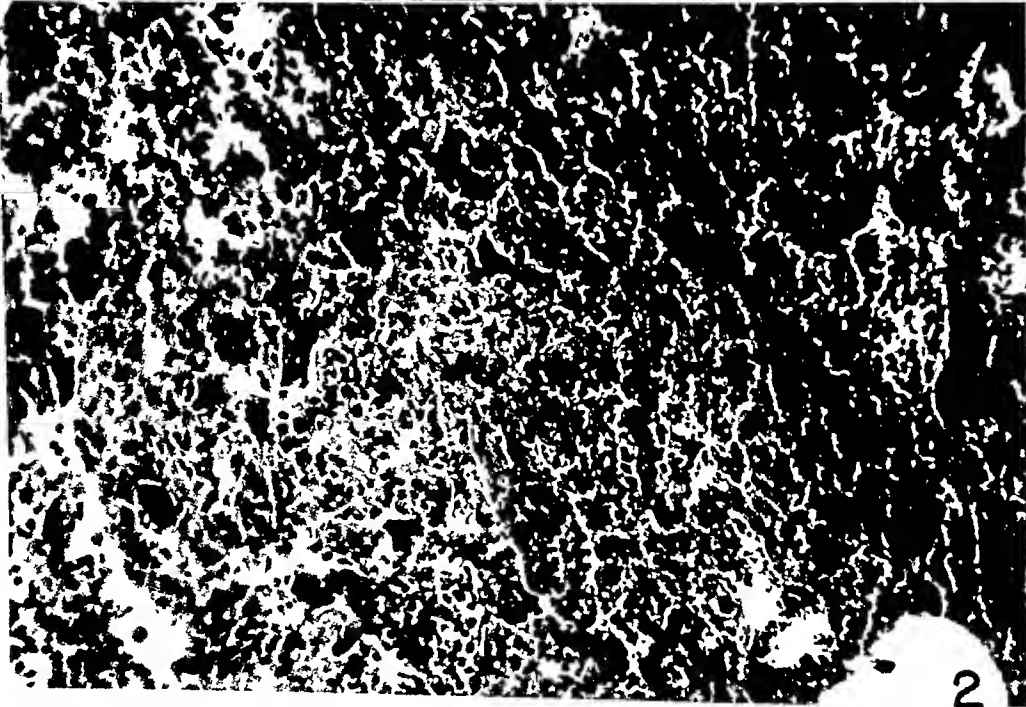
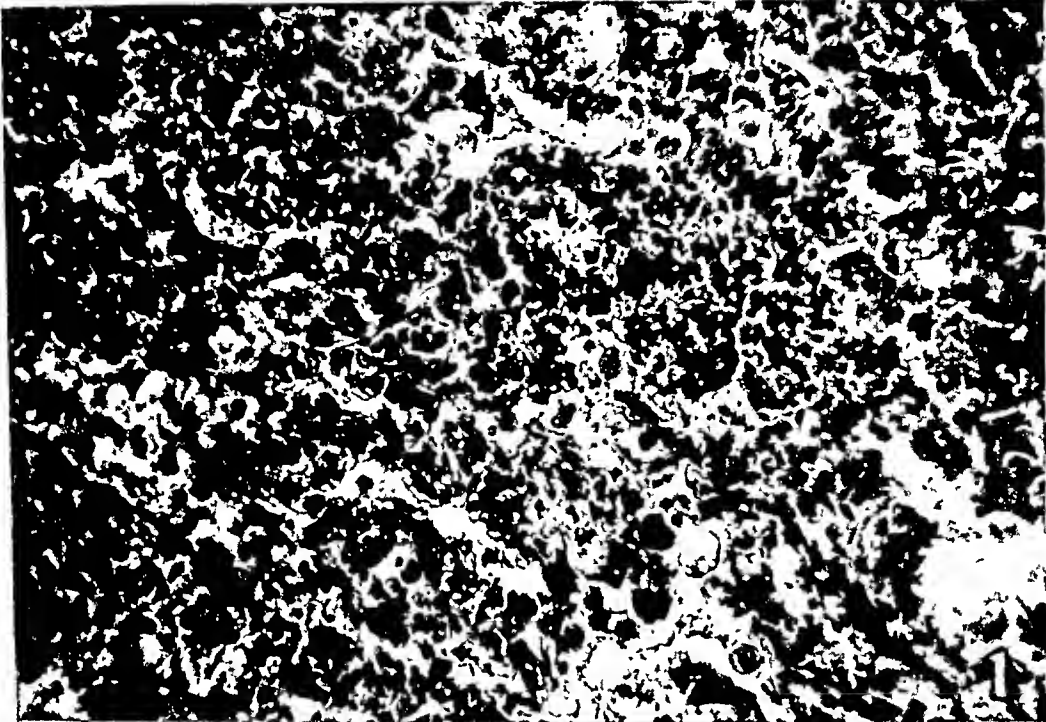
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DESCRIPTION OF PLATES

PLATE 31

- FIG. 1. Section of the lung of a rabbit receiving pertussis toxin but no antitoxin. This animal was killed 96 hours after injection of the toxin. This reaction was listed as a 3 plus reaction and shows marked proliferation of mononuclear elements. Hematoxylin and eosin stain. $\times 200$.
- FIG. 2. Section of the lung of a rabbit receiving pertussis toxin but no antitoxin. This reaction was listed as a 4 plus reaction. An area of necrosis may be seen. Hematoxylin and eosin stain. $\times 400$.



Sprunt and Martin

Neutralization of Pertussis Toxin

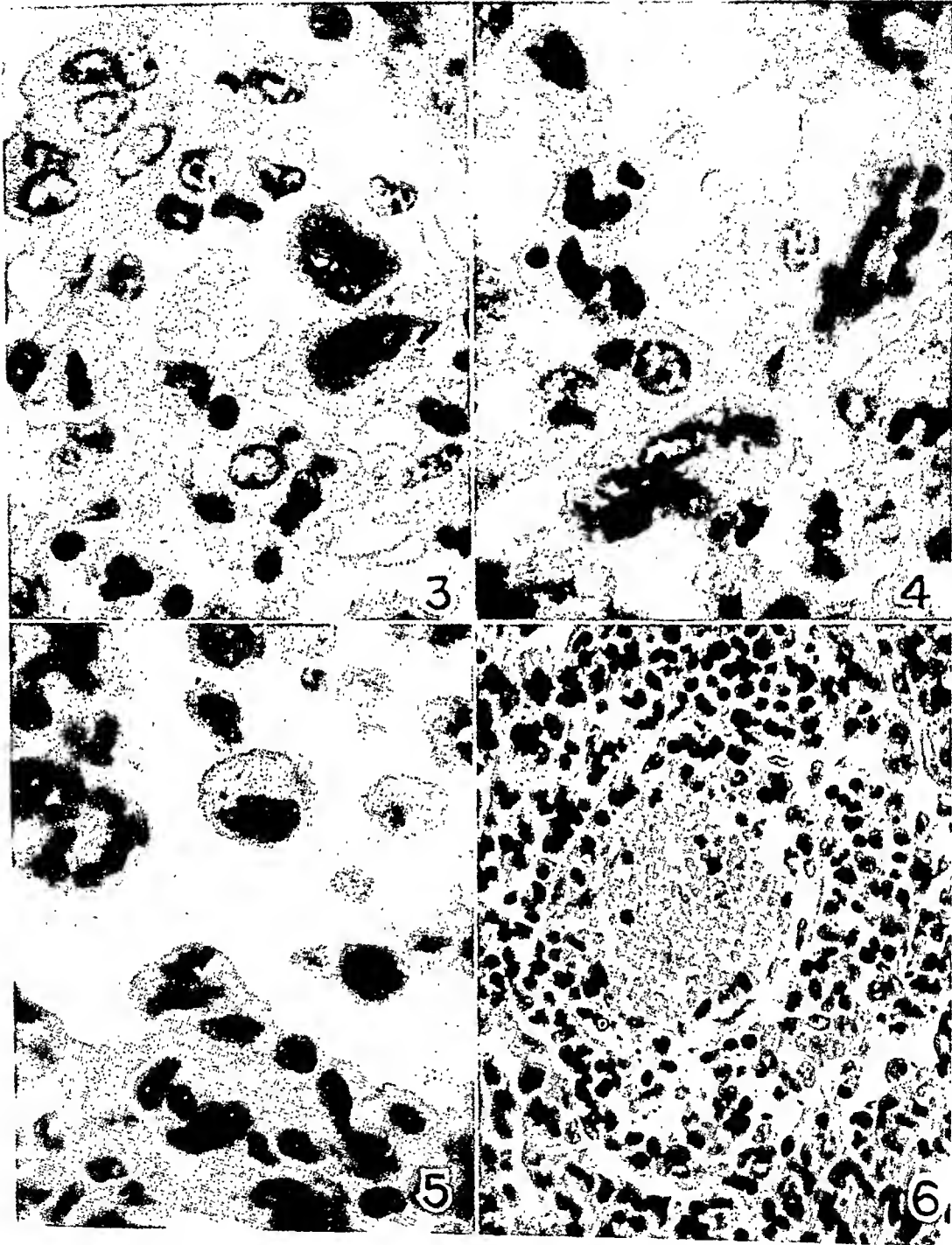
PLATE 32

All figures were taken from the same section as Figure 1.

FIGS. 3 and 4. These areas show thickening of the alveolar walls and mononuclear cells in the lumina. Hematoxylin and eosin stain. $\times 900$.

FIG. 5. In this area there are macrophages in an alveolus. Hematoxylin and eosin stain. $\times 900$.

FIG. 6. A perivascular infiltration of lymphocytes is shown in this area. Hematoxylin and eosin stain. $\times 400$.



Sprunt and Martin

Neutralization of Pertussis Toxin

THE EFFECT OF CRYSTALLIZED BOVINE SERUM ALBUMIN ON THE TISSUES OF NORMAL ANIMALS

I. MORPHOLOGIC CHANGES IN NORMAL RABBITS INDUCED BY INTRAVENOUS INJECTION OF CRYSTALLIZED BOVINE SERUM ALBUMIN*

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The methods for the fractionation of plasma proteins recently developed in the Department of Physical Chemistry of the Harvard Medical School have made available the proteins of blood plasma in states of great purity and in far larger amounts than could readily have been yielded by previous methods. The use of these materials has provided a new approach to many problems in biology and in clinical medicine.¹⁻⁸

Of the proteins prepared by the new technic, the albumin of human plasma has been made available in considerable amounts by Cohn, Strong, Oncley and Armstrong, and has already been used in the clinic, especially in the treatment of shock.^{3, 5-8} Because of the necessity of obtaining large quantities of material for use in the treatment of shock, a substitute for human serum albumin is required. Cohn and Hughes have prepared crystallized albumin from bovine plasma.

Before using bovine serum albumin as a therapeutic agent in man, it seemed advisable to determine its effect on animals. Biologic agents produced from one species of animals may lead to different results when injected into heterologous animals of different species. In spite of this, the effects of bovine serum albumin on experimental animals may serve as a useful guide in determining the suitability of this material for human therapy. The present report is devoted to a study of the consequences of intravenous injection of bovine serum albumin in the rabbit.

Bovine serum albumin injected in various species of animals and in man is not excreted as such by the kidneys. Since the albumin does not appear in the urine and no other method of excretion has yet been

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found, it is presumed to be dealt with by the organism receiving the injection. The albumin might be used in metabolism, or it might be stored, with or without chemical modification. It might or might not produce, in various organs, changes which would serve as contraindications for its use therapeutically. These problems can best be studied in normal animals, in which tissue alterations not due to the albumin are at a minimum.

Rabbits were chosen as the first experimental animals to be studied because of the ease of intravenous injection, because they belong to an entirely different species than that from which the albumin was obtained and because the normal variations in histology are well known. Other species of animals have been used, with results for the most part similar to those in rabbits. These experiments will be discussed in subsequent reports.

The experiments with crystallized bovine serum albumin have been designed to determine the effect of a single intravenous injection of a size comparable, per Kg. of body weight, to that used in man; of repeating this injection once, and of repeating the injection several times.

MATERIALS AND METHODS

Properties of the Crystallized Bovine Serum Albumin

Crystallized bovine serum albumin in the solid state is a white powder composed of crystals of various shapes. The molecular weight as determined by osmotic pressure is 70,000. A comparable figure for the molecular weight has been obtained by using the ultracentrifuge. The impurities detected by the methods available are less than 0.5 per cent. The crystallized bovine albumin under consideration is readily soluble in water, yielding clear solutions even at concentrations greater than that used in the animal experiments (25 per cent).

For the purposes of the experiments described in this report, 25 gm. of the crystallized bovine serum albumin was dissolved in 100 cc. of water especially distilled for intravenous use. The solution was passed through a Seitz filter (D-2 pad). Sodium bicarbonate (0.375 gm. per 100 cc. solution) and sodium chloride (0.55 gm. per 100 cc. solution) were added. The solution was then sterilized by filtration through a Seitz filter (D-8 pad) and bottled in sterile containers. No merthiolate or other preservative was added.

Experimental Procedure

Twenty-one rabbits were injected with crystallized bovine serum albumin in the ear veins. The material was used in 25 per cent solution. Each dose consisted of 1 gm. (4 cc.) per Kg. of body weight. Twenty

animals survived for the duration of the experiments. Their general condition remained excellent, no loss of weight developed and no abnormalities of behavior were noted. One rabbit received seven injections in 14 days, during which it remained in good condition. On the second day after the seventh injection, the rabbit appeared sick and death occurred on the third day. Autopsy showed that death was due to bronchopneumonia. Since considerable postmortem change had taken place in the organs, the tissues from this animal were not used for detailed study.

The 20 rabbits in the series received crystallized bovine serum albumin (1 gm. per Kg. of body weight) as follows:

- 8 received a single injection and were sacrificed after 2, 6, 24, 48 (2 animals) and 72 hours, and 7 and 15 days;
- 4 received two injections 48 hours apart and were sacrificed 24, 48 and 72 hours, and 11 days after the second injection;
- 6 received seven injections in 15 days and were sacrificed 1, 4, 6, 9 and 18 (2 animals) days after the last injection;
- 2 received twelve injections in 25 days and were sacrificed 5 and 28 days after the last injection.*

The rabbits were killed with an overdose of chloroform and autopsied immediately. Rabbits kept under the same conditions, fed the same diet and sacrificed in the same way, but receiving no injections, were used as controls.

Thin sections of all organs were fixed as soon as possible in large quantities of Zenker's fluid and in 4 per cent solution of formaldehyde in physiologic saline. The tissues fixed in Zenker's fluid were embedded in paraffin and stained with eosin and methylene blue or phloxine and methylene blue. Sections of the spleen and kidney in several instances were also stained by the Turnbull's-blue method for iron. Portions of the spleens of two animals were fixed in corrosive sublimate and acetic acid for Feulgen's stain.⁹ This technic, however, gave better results on tissues fixed in Zenker's fluid. For this reason, Zenker's-fixed tissues were used by preference for studies by Feulgen's method. Frozen sections cut from formaldehyde-fixed tissue were stained with scarlet red for the demonstration of fat.

Effect of Albumin Injections on Blood Count

A rabbit received one intravenous injection of bovine serum albumin; the red blood cell count, white blood cell count and differential count were made before injection and at half-hour intervals for 6½ hours. No significant variation was found in any of the counts.

* This would correspond to a total dosage of 840 gm. in a man weighing 70 Kg.

Two rabbits were given seven intravenous injections of bovine albumin in 15 days; blood counts were made after the second and each subsequent injection and at intervals of 4 days for 20 days after the last injection. No significant variation developed in the red blood cell count, white blood cell count or differential count.

TISSUES SHOWING LITTLE OR NO ALTERATION

Heart. The myocardial fibers were normal in all rabbits. In 4 of the rabbits (2 of those receiving one injection and 2 receiving seven injections) the number of lymphocytes and mononuclear cells in the myocardial connective tissue was slightly greater than that seen in the control rabbits. Since the same types of cells were found in varying numbers in the myocardial connective tissue of the controls, no particular significance was attached to this observation.

Lung. In 12 rabbits (4 with one injection; 2 with two injections; 6 with seven injections) there were small fresh hemorrhages without inflammatory reaction. Such small fresh hemorrhages are frequently seen in the lungs of rabbits sacrificed after many types of experimental procedures. There was no correlation between dosage of albumin and their occurrence, or between them and the interval separating the time of the last injection and the time of autopsy. No hemosiderin deposits or other evidences of old hemorrhage were found in any of the animals. For these reasons, we do not feel that the hemorrhages are related to the injections of bovine serum albumin. The trachea was normal in all rabbits in the series.

Gastrointestinal Tract. The esophagus, stomach, small intestine and colon were examined. These organs were normal in all rabbits.

Pancreas. Normal in all animals.

Adrenals. There were very small numbers of polymorphonuclear leukocytes scattered in the connective tissue of the adrenal cortex in 3 animals. No necrosis of the cortical parenchyma was found. The medulla was normal in all instances.

Ureter. Normal (examined in 2 animals).

Urinary Bladder. Normal in all animals.

Testis. Nineteen of the rabbits were males. The testes of 7 of these showed slight evidence of vitamin E deficiency. Similar changes were present in some of the control animals. These alterations are therefore regarded as the result of insufficient alpha-tocopherol in the diet rather than the consequence of the injections of bovine serum albumin. There were no testicular changes which could not be accounted for in this way.

Ovary and Uterus. These organs were normal in the 1 female rabbit in the series.

Thyroid. The thyroid was examined in 6 rabbits (1 which received one injection; 1, two injections; 3, seven injections; 1, twelve injections). It was normal in each instance.

Thymus. The thymus was negative in 6 rabbits (2 which received one injection; 1, two injections; 2, seven injections; 1, twelve injections).

Aorta. The aorta was normal in all rabbits of the series.

Abdominal Skin. Negative in all animals.

Musculature. The psoas muscle and muscles of the abdominal wall were examined in each rabbit. These were normal in all instances.

Central Nervous System. The cerebral cortex, hippocampus, brain stem, cerebellum and spinal cord were examined routinely. These levels of the central nervous system were normal in all the rabbits receiving one injection. All 4 rabbits to which two injections had been given showed a very small number of polymorphonuclear leukocytes in the central nervous system, unaccompanied by necrosis of cerebral tissue. In the rabbit autopsied 24 hours after the second injection, the cells were found at all levels examined. They were present in each of the sections except that of the spinal cord in the animal sacrificed 48 hours after the second injection. In the rabbit autopsied after 72 hours, there were increased numbers of polymorphonuclear leukocytes within the blood vessels of the cerebral cortex, hippocampus and brain stem, but none in the brain tissue itself. A very few cells of this type were found in the interstitial tissue of the cerebral cortex and hippocampus of the rabbit sacrificed 11 days after the second injection. There were no similar changes in any of the rabbits receiving seven injections or those with twelve injections. The rabbit receiving seven injections and autopsied 9 days after the last injection showed the lesions of spontaneous encephalitis of rabbits.

TISSUES WITH CHANGES ASSOCIATED WITH INCREASED DESTRUCTION OF BLOOD CELLS

Spleen. The spleen was slightly to moderately enlarged in each of the animals of the series autopsied within 10 days after cessation of the injections. In rabbits autopsied later the spleens showed no significant deviation from the normal on gross inspection.

The changes in the spleen have been interpreted as the result of an increase in phagocytosis of leukocytes and red blood cells in the splenic pulp. The differences between the spleens of the animals receiving injections of crystallized bovine serum albumin and those of normal controls are quantitative rather than qualitative.

In the spleen of the rabbit sacrificed 2 hours after a single injection of albumin, polymorphonuclear leukocytes in increased number were

found scattered through the pulp. Most of these cells were normal in morphology but many had nuclei which took the nuclear stain less deeply than usual. Occasionally, polymorphonuclear leukocytes were found in phagocytic cells. Both eosinophilic and neutrophilic varieties were affected. Fragments in the form of small, round, densely basophilic droplets were found in the pulp in moderate numbers. The droplets were, for the most part, in phagocytic cells, but some of them appeared free in the meshes of the pulp. Occasionally, the droplet-filled phagocytes were present lining the sinusoids and within their lumina. These droplets, whether intracellular or extracellular, were mostly grouped in foci; these were more abundant in the zone beneath the capsule than in the central portions of the pulp. By study of many microscopic fields, it was possible to find transitional stages between the droplets and the nuclei of degenerating polymorphonuclear leukocytes. The cytoplasm disappeared first and left no demonstrable trace. The granules of eosinophils often persisted longer than other cytoplasmic constituents. A few nuclei of polymorphous shape without cytoplasm were found in phagocytes.

Additional evidence that the basophilic droplets were of nuclear origin was afforded by study of sections stained by Feulgen's technic. Feulgen's method stains thymonucleic acids purple. Since thymonucleic acids occur in living tissues only in chromatin, the technic is specific for nuclear material. The droplets under consideration were stained purple by this method and the transitional stages were especially well shown. Similar droplets were also present in the spleens of normal control rabbits (Fig. 1). We have interpreted these findings as indicating that the rate of destruction of leukocytes, normally taking place in the rabbit spleen, was somewhat increased by the intravenous injection of crystallized bovine serum albumin and that this increase was already evident 2 hours after the time of injection.

The spleens of the rabbits sacrificed 6, 24 and 48 hours after a single injection showed progressive stages in the degeneration of polymorphonuclear leukocytes, apparently without further increase in the number affected. The partly degenerated, but partially intact, leukocytes were less and less frequent, deeply basophilic droplets being found in their stead. Seven days after injection there were only a few partially degenerated polymorphonuclear leukocytes, but the droplets were still present in considerable excess over the numbers in the normal spleen. Partial digestion of the droplets of nuclear material in the phagocytes was suggested by further fragmentation and decrease in size. In the rabbit sacrificed 15 days after a single injection, the quantities of degenerating polymorphonuclear leukocytes and of basophilic

droplets were essentially those of the spleens of normal control rabbits.

Phagocytosis of red blood cells was not increased to a significant degree in the spleens of rabbits sacrificed 2 and 6 hours after a single injection. This process, however, was conspicuous in the animals autopsied after 24 hours. Red blood cells, singly or in groups, were found in phagocytic cells of the pulp and in the sinusoids. Phagocytosis of red blood cells was present also in the spleens of animals sacrificed at 48 hours, 72 hours and 7 days.

Small amounts of hemosiderin were present in the spleen of the rabbit sacrificed 7 days after a single injection. This material was found in phagocytic cells, usually in the pulp but occasionally in the sinusoids. A few hemosiderin-filled phagocytes were seen in the spleens of normal control rabbits, but the increase in the experimental animal was definite. The amount of hemosiderin in the spleen of the rabbit autopsied 15 days after a single injection was less than it was in the animal sacrificed after 7 days but it was still considerably above the upper limit of normal. The hemosiderin appeared to be derived from degeneration of the phagocytosed red blood cells.

The same cells were concerned in the phagocytosis of red blood cells and of polymorphonuclear leukocytes. Some contained only red blood cells, others only degenerating leukocytes; at times, a single phagocyte contained both, or their degeneration products.

There was no definite change in the size, architecture, or cellular composition of the malpighian corpuscles of the several animals sacrificed after a single injection of albumin. The central arterioles, veins, trabeculae and capsule showed no essential variation from the normal. There was a variable degree of dilatation of the sinusoids. Except for the sequences described previously, the cellular composition of the pulp varied only within limits seen in normal control animals.

The spleens of the animals which received two, seven and twelve injections of crystallized bovine albumin showed changes differing only in degree from those already described (Figs. 2, 3 and 4). Separation of the several stages in the degeneration of polymorphonuclear leukocytes and red blood cells was rendered less clear in the rabbits receiving multiple injections of albumin. This was interpreted as an indication that each separate injection led to the degeneration of additional cells of both types. Thus, when a rabbit was sacrificed 24 hours after the last of seven injections, some of the stages of blood cell phagocytosis resembled those seen 24 hours after a single injection. Other stages corresponded to those found in rabbits autopsied at longer intervals after administration of albumin. In addition to the sequences connected with blood cell degeneration, there was a definite but moder-

ate hyperplasia of the malpighian corpuscles in the spleens of rabbits receiving seven and twelve injections of albumin (Fig. 5). These structures showed active proliferation of the cells in the germinal centers. In spite of the additive character of the changes resulting from several injections of albumin, the spleens again returned to normal after a few weeks. For instance, in the spleen of the rabbit sacrificed 28 days after the last of twelve injections, occasional hemosiderin-filled phagocytes were the only traces of blood cell phagocytosis.

In summary, the changes in the spleen are those of quantitative increase over the normal rate of blood cell destruction in this organ. Within a few weeks, the sequences progress to complete disappearance of the degeneration products of leukocytes and red blood cells. The changes in the spleen induced by injections of crystallized bovine albumin are, therefore, entirely reversible.

Mesenteric Lymph Nodes. The lymph nodes of rabbits, especially those of the mesentery, presented considerable variation in size, amount of lymphoid tissue and degree of sinusoidal dilatation under normal conditions. The mesenteric lymph nodes of the rabbits in the present series showed variations which were not beyond those of a similar group of control animals. These variations could not be correlated with the number of injections or the interval between the last injection and the time of autopsy.

Within and around the sinusoids, the same sequences were seen as in the spleen. There was phagocytosis of polymorphonuclear leukocytes with progressive disintegration, nuclear fragments persisting longer than the rest of the cell. Red blood cells were also phagocytosed and hemosiderin appeared as the result of their breakdown. The time relationships, including the time of return to normal, were the same in the lymph nodes as in the spleen. This process of phagocytosis was more extensive in the spleen than in the lymph nodes. In the few instances in which lymph nodes other than the mesenteric were examined, identical sequences were found.

Liver. There was coccidiosis in the livers of 6 rabbits. Otherwise, the general architecture of the liver was entirely normal. No changes suggesting storage of albumin were found.

Phagocytosis of polymorphonuclear leukocytes and red blood cells by Kupffer cells occurred to a limited extent. By careful search, evidence of such phagocytosis could be found in the Kupffer cells in normal control rabbits. Here, again, the differences between normal organs and those of the experimental animals were quantitative rather than qualitative. The sequences were identical with those described in the spleen. Phagocytosis of polymorphonuclear leukocytes was

noted earlier than that of red blood cells. In the animals allowed to survive longer after injection, nuclear fragments and hemosiderin made their appearance; these were not increased over the normal in animals sacrificed at the longest times after cessation of injections.

Bone Marrow. There was no definite change in the cellularity of the marrow in rabbits receiving one, two and seven injections.

A slight increase in cellularity of the marrow was present in the animal examined 5 days after the last of twelve injections (compare Figs. 6 and 7). The proportion of adult polymorphonuclear leukocytes was slightly increased in the marrow of rabbits receiving seven and twelve doses. In the rabbit sacrificed 28 days after the last of twelve injections, the marrow was normal in regard to number and character of cells.

Phagocytosis of adult blood cells occurred in the marrow of several rabbits to a very slight extent, the sequences being the same as those already described for the spleen, Kupffer cells and lymph nodes. Small amounts of nuclear debris and hemosiderin made their appearance as the result of blood cell degeneration, but these were not found in the animals allowed to survive longest after completion of the injections.

The Kidneys

The changes in the kidneys of rabbits injected with bovine serum albumin differ from those concerned with the degeneration and phagocytosis of polymorphonuclear leukocytes and red blood cells. In the kidneys of animals given a single injection of albumin and sacrificed at the various intervals listed under *Materials and Methods*, the glomeruli, blood vessels, collecting tubules, pelvic epithelium, renal capsule and stroma were entirely normal in appearance. There was slight swelling of the cells lining the ascending limbs of Henle's loops and the adjacent portions of the distal convoluted tubules, with dispersion of the cytoplasm. This swelling and concomitant dispersion of the granular cytoplasm was present to a degree only slightly beyond that seen in some areas of the kidneys of normal controls. It was evident in the kidneys of rabbits sacrificed 24, 48 and 72 hours after injection. The distribution of these minimal changes was fairly uniform; they were found to an equal degree in the central portions and at the poles. The lumina of the tubules contained very small amounts of granular precipitate similar in appearance and amount to that seen in controls. The other portions of the renal tubules in these rabbits were free from change. The kidneys of animals sacrificed at 2 hours, 6 hours and 15 days following a single injection showed no essential deviation from the normal.

In the kidneys of the rabbits sacrificed 24 hours after the second of two injections, there was a slight to moderate swelling of the tubular cells and a somewhat greater dispersion of the cytoplasm than was seen after one injection. These changes involved the ascending limbs of Henle's loops, the adjacent portions of the distal convoluted tubules and, in addition, portions of the proximal convoluted tubules.

Examination of the kidneys of the rabbit autopsied 48 hours after the second of two injections of albumin showed that there was further swelling of the same cells as were affected in the rabbit sacrificed after 24 hours. In several small areas, the swelling and dispersion of the cytoplasm resulted in the formation of large clear cells with a narrow rim of cytoplasm and the nucleus basally placed. The large central portion of these cells was entirely clear; the vacuoles did not contain fat in sections stained with scarlet red. A few fine droplets at the bases of the cells were colored red by this method.

The enlargement of these cells resulted in marked narrowing or even obliteration of the lumina of the tubules lined by them. The transition from slightly swollen tubular cells to the large clear cells was usually abrupt.

Evidence that the processes leading to the appearance of the large clear cells do not lead to necrosis of the cells was found in the absence of nuclear degeneration and of mitotic figures in the adjacent tubular epithelium. No leukocytic infiltration was seen in association with these cells. The absence of these cells with clear cytoplasm in animals allowed to survive longer after the last injection of albumin suggested that the process was reversible.

Changes similar in appearance, degree and frequency to those in the rabbit autopsied at 48 hours after the second injection were seen in the animal sacrificed after 72 hours. The appearance of the tubular epithelium in the rabbit autopsied 11 days following the second of two injections was essentially that of the control animals.

In all of the animals receiving two injections of crystallized albumin, the tubules contained very small amounts of granular precipitate, similar to that seen in control rabbits. The glomeruli, stroma, blood vessels, collecting tubules, pelvic epithelium and renal capsule were normal.

The kidneys of the animals sacrificed at various intervals after seven injections showed changes of essentially the same character as those described in the preceding series of rabbits, the differences being those of degree. Those seen in the animal autopsied 24 hours after the seventh injection were moderate to marked swelling of the cells lining nearly all ascending limbs of Henle's loops as well as those of the

distal convoluted tubules, descending limbs of Henle's loops and, to a less degree, the proximal convoluted tubules (Fig. 9). There were hyaline casts in the lumina of occasional tubules, for the most part in the descending limb of Henle's loop; there were a few in the collecting tubules. The amount of granular precipitate in the proximal and distal convoluted tubules was considerably increased over the amount seen in normal control rabbits. A small amount of granular precipitate was seen also in the lumina of some collecting tubules.

In the rabbit autopsied 4 days after the last of seven injections there were changes qualitatively similar to those just described, but markedly less in degree. Relatively few of the cells lining the tubules showed the clear cytoplasm with basally placed nucleus. The tubular cells were, on the average, smaller than equivalent cells in the animal sacrificed 1 day after the last of seven injections. The amount of granular precipitate in the convoluted tubules and in Henle's loops was less than in the rabbit autopsied at 24 hours, but was still in excess of that seen in control animals. No casts were present.

There were scars in the kidney of the rabbit sacrificed 9 days after the last of seven injections. These scars were not seen in any of the other animals of the series; they can be said with certainty to have antedated the experiments. Where uninvolved by the scarring, the renal tubules showed no significant differences from those of normal control animals.

The kidneys of both rabbits sacrificed 18 days after the last of seven injections showed a few groups of swollen cells with clear cytoplasm and basally placed nuclei in the distal convoluted tubules and ascending limbs of Henle's loops. The remainder of the tubules were normal in appearance. The glomeruli, blood vessels, stroma, collecting tubules, pelvic epithelium and renal capsule were normal in the animals receiving seven injections, except for the already described areas of scarring in 1 rabbit.

One of the 2 animals receiving twelve injections was sacrificed 5 days after the last injection. There were infrequent segments of the distal convoluted tubules or adjacent limbs of Henle's loops in which clear swollen cells were present. These changes were identical with those previously described; the general appearance of the kidney was similar to that of the kidney in the rabbit sacrificed 6 days after the last of seven injections. In the kidney of the animal autopsied 28 days after the last of twelve injections, there were very rare groups of clear cells in the tubules (not more than three in a section including the entire kidney). Except for these groups of cells, the kidneys were entirely normal (Fig. 10).

DISCUSSION

This study has been designed primarily to determine whether crystallized bovine serum albumin injected intravenously in rabbits is stored in tissues for considerable periods of time, whether there are morphologic changes in the tissues of the experimental animals which can be attributed to the injections of albumin and whether the consequences of repeated injections are different from those of one injection. In addition, in this study we have attempted to determine whether crystallized albumin may be used for investigation of the biology of tissues.

Crystallized bovine serum albumin is not stored for any considerable period of time in the tissues of the rabbit in such form as to be recognized by morphologic methods. In this, albumin differs from solutions of many other compounds of high molecular weight, such as gum acacia and polyvinyl alcohol.¹⁰ Since the bovine albumin is not excreted in the urine but disappears slowly from the circulating blood without being stored as such in the tissues, it is presumed to be used up in metabolism or to be incorporated, either with or without change, in the structure of protoplasm. The technics used in this study are not sufficient to distinguish between these possibilities.

The general condition of the animals remained excellent throughout the experiments. Weight was maintained and no abnormalities of behavior developed. Repeated injections, up to twelve, had no more effect on the general condition of the rabbits than did a single injection. The relation of multiple injections to tissue reactions is discussed elsewhere. It should be emphasized that the amounts of albumin per kilogram of body weight were large—up to 12 gm. per Kg. This is in excess of the amount likely to be required in the treatment of human patients and is also greater than the quantity necessary to produce striking tissue changes with gum acacia, polyvinyl alcohol and other large-molecular compounds.¹⁰

The morphologic changes in the tissues of rabbits receiving intravenous injections of bovine serum albumin fall into two groups. The first group consists of changes in the spleen and other organs of the reticulo-endothelial system; the second group is concerned with alterations in the kidneys.

The histologic changes in the spleen and to a less degree in the liver, lymph nodes and bone marrow depended upon tissue sequences concerned with the degeneration and phagocytosis of red blood cells and polymorphonuclear leukocytes. This process was seen in a small number of cells in the same organs of normal rabbits and the sequences are identical in the control and in the experimental animals. For this

reason, the alterations are interpreted as the result of a quantitative increase in the rate of a normal process.

MacKenzie, Whipple and Wintersteiner¹¹ have shown that the interstices of the splenic pulp act as a filter separating blood cells from the plasma. Some cells of the blood pass rapidly through the meshes of the pulp, while others remain there for considerable periods of time, as in eddies alongside a swiftly moving current. For this reason, part of the blood cells are exposed to the phagocytes of the splenic pulp much longer than are others. The phagocytes under normal conditions take up a few red blood cells and leukocytes. These cells disintegrate in the phagocytes. Hemosiderin is produced from the degenerated red blood cells (Fig. 2) and nuclear fragments persist after the other portions of leukocytes can no longer be identified morphologically (Figs. 3 and 4).

The intravenous injection of crystallized bovine serum albumin in rabbits leads to increased destruction of blood cells in the spleen. This increase is noted first in the case of the polymorphonuclear leukocytes, being evident in the animal sacrificed 2 hours after a single injection. Phagocytosis of red blood cells was not definitely increased over the normal in rabbits autopsied 2 and 6 hours after a single injection but was demonstrated 24 hours after injection. We have not been able to determine as yet the actual mechanism by which intravenous injection of heterologous albumin leads to an increase in the amount of normal blood cell phagocytosis in the spleen. Studies on normal human subjects indicate that there is a very rapid adjustment to the physiologic changes brought about by the intravenous injection of crystallized bovine albumin. These studies have not been repeated on the rabbits in this series. However, it seems logical to assume that the increase in osmotic pressure caused by the albumin is compensated within a few minutes in the rabbit, as in man. Since histologic evidence of red blood cell destruction in abnormal amounts is not found for several hours after administration of albumin, it seems unlikely that the changes in osmotic pressure or in blood volume are directly related to the increase in rate of phagocytosis of red blood cells. A more probable explanation, but one not established by this study, is that the intravenous injection of bovine albumin in rabbits produces flocculation of relatively small numbers of red blood cells which are then separated from the plasma in the splenic pulp and broken down over a period of time.

Since increased phagocytosis of leukocytes appears at a significantly earlier time after injection of bovine albumin than does phagocytosis of red blood cells, the degeneration of leukocytes may depend either

upon a different mechanism from that affecting the red blood cells or the leukocytes may succumb more quickly to a similar alteration. The leukocytes of the rabbit are known to be affected more easily by a number of pathogenic agents than are the leukocytes of several other species of animals commonly used in the laboratory.

Sequences of phagocytosis of red blood cells and leukocytes were also found to a much smaller extent in the mesenteric lymph nodes, bone marrow and Kupffer cells of the liver. They would appear, therefore, to be concerned with the reticulo-endothelial system or a considerable number of its components. The increase in rate of blood cell phagocytosis, while definite, does not affect a large proportion of the blood cells, as shown by the fact that counts on the circulating blood do not change significantly. There is only a slight hyperplasia of the bone marrow, even after twelve injections.

When a series of injections of bovine albumin are given intravenously, each injection initiates a new set of the same sequences. The tissues of rabbits autopsied after several injections show some sequences near the final stage of disappearance of hemosiderin and nuclear particles and others at the stage of fresh phagocytosis of blood cells. With the cessation of injections, the sequences go on to conclusion and the organ returns to the state in which it was before albumin has been administered. The changes induced in the spleen and other organs of the reticulo-endothelial system are entirely reversible, so far as each organ as a whole is concerned. The changes in the individual phagocytosed cells are, of course, irreversible.

The histologic sequences in the kidneys of rabbits receiving bovine albumin are different from the sequences found in the spleen and other organs of the reticulo-endothelial system. The study of the morphologic changes in the kidney is best attacked by considering some of the facts of renal physiology. It has been demonstrated¹²⁻¹⁴ that there is a renal threshold for such relatively large molecules as hemoglobin (molecular weight, 68,000). A state of equilibrium exists between the amount of hemoglobin absorbed by the tubular epithelium and the amount of hemoglobinogenous products removed from these cells per unit of time.¹² Direct measurements of the nature and amount of the protein in glomerular filtrates have been made.^{15, 16} It is important to bear in mind that the presence of small quantities of protein in the glomerular filtrate, as noted in the literature, actually indicates that relatively large quantities of protein are filtered through the glomerulus and reabsorbed by the tubules when the amount of glomerular filtrate is compared with the amount of urine excreted.

These facts aid in understanding the morphologic changes in the

renal tubular epithelium as the result of intravenous injection of crystallized albumin. The studies on hemoglobin absorption are especially helpful since the molecular weight of hemoglobin is of the same order of magnitude as that of bovine albumin. The alterations noted histologically in the rabbits receiving bovine albumin involve the swelling and dispersion of the cytoplasm of the epithelium of the ascending limb of Henle's loop and the adjacent distal convoluted tubules. The most marked degree is found in animals sacrificed soon after several injections; in these animals the cytoplasm appears clear in tissue sections and the nucleus is displaced toward the basement membrane (Fig. 9). After a single injection, the changes are maximal between 24 and 72 hours; thereafter, they are regressive. Crystallized bovine serum albumin does not appear in the urine when injected intravenously in human patients and in dogs; we have not tested the urine of the rabbits used in this study.

The swelling and dispersion of the cytoplasm seem to be related definitely to the injections of bovine albumin. They are not found in the normal rabbit kidney; their degree can be directly correlated with the number of injections and the interval after the last injection. The changes appear to represent absorption rather than excretion. Confirmation of this view is found in the kidney showing maximal changes in tubular epithelium, and hyaline casts in addition. These casts were, for the most part, in the descending limb of Henle's loop and not in the ascending limb, convoluted tubules, or collecting tubules. It might be expected that casts would appear in, and distal to, the ascending limb of Henle's loop if the lining cells were excreting protein, rather than absorbing it. We feel, then, that some of the crystallized bovine albumin passes the glomerular filter in the rabbit's kidney at a rate comparable to that observed for such proteins as hemoglobin; it is then absorbed by the tubular epithelium and returned to the circulation, with or without chemical modification.

Particular attention has been paid to the condition of the aorta and large arteries of the organs in all the animals in view of Hueper's ¹⁰ studies on the relation of certain compounds of high molecular weight to vascular lesions. Hueper ¹⁷ administered polyvinyl alcohol intravenously and intraperitoneally in 5 per cent solution to dogs and found that atheromatous lesions in the aorta, carotid and femoral arteries resulted. This material was also found within the media of smaller arteries and arterioles of the heart and kidney. He regarded the lesions as morphologically similar to the cholesterol atheromatosis observed in man and rabbits. From these and additional experiments ^{18, 19} he concluded that the presence within the blood and tissues of abnormal

amounts of chemically inert macromolecular substances, which resist metabolic degradation and are therefore not readily eliminated through the filtration membranes, leads to the development of organic and particularly vascular lesions which display a certain degree of similarity in location and character.¹⁰

Study of the animals in this series indicates that such changes do not result from the intravenous administration of crystallized bovine albumin. The experimental animals in Hueper's experiments received amounts of polyvinyl alcohol per kilogram of body weight which are of the same order of magnitude as the amounts of crystallized albumin used in our experiments. Comparable time intervals were used in both sets of experiments. The actual excretion of crystallized albumin or of the products of breakdown of this substance has not been demonstrable. Since the albumin has been found to disappear from the blood stream slowly over the course of several days, it must be considered to be utilized in metabolism or stored. The absence of atherosclerosis in all of our experimental animals and, indeed, the absence of histologic evidence of deposition or storage of the albumin or of the products of disintegration of this substance are evidence that the prolonged presence of this exogenous macromolecular substance does not give rise to any of the changes which might be expected from it if all macromolecular substances were to give rise to the morphologic changes due to methyl cellulose and polyvinyl alcohol. The experimental evidence indicates that crystallized bovine serum albumin, although an exogenous material in the rabbit, is dealt with by the tissues in far different fashion from the macromolecular compounds studied by Hueper.¹⁰ The size of the molecule is a secondary factor in determining the character, distribution and extent of tissue reaction to exogenous materials.

In view of Letterer's^{20, 21} theory that amyloid is produced by a local reaction of antigen and antibody, we have searched for this material in the rabbits injected with crystallized bovine serum albumin. It has not appeared in any instance. The intervals during which the animals have been observed are not so long as would be useful in excluding completely the possibility of the formation of amyloid. Studies have been undertaken, to be reported in detail later, on the effect of very large amounts of crystallized bovine serum albumin in mice. The mouse is an experimental animal in which amyloid is produced quickly by a variety of agents, yet preliminary studies indicate that amyloid is not produced under these experimental conditions.

When the present study was begun, amorphous albumin containing a low percentage of other blood proteins was the purest preparation

available. Within a short time, crystallized albumin was prepared; the experiments were repeated with this material. Comparison of the tissues of animals injected with the two types of preparation indicates that the histologic changes resulting from amorphous bovine albumin were greater and, to a certain extent, different from those elicited by the crystallized material.

The availability of crystallized albumin furnishes a new biologic technic for study of many problems in tissue behavior. This study, undertaken from another point of view, has suggested that it may be used in investigation of problems in connection with the physiology and pathology of the spleen and kidney. Its value is enhanced by the fact that other blood proteins in highly purified form may be used in conjunction with the albumin or in comparison with it.

It is evident from these experiments that large and repeated intravenous injections of crystallized bovine serum albumin produce relatively minor histologic changes in the rabbit; the changes which are produced are reversible. In some of the animals, the total amount of albumin injected was approximately twice that of the total protein of the circulating blood of the rabbit. The use of crystallized bovine serum albumin in the rabbit involves a species difference between the animal from which the material was derived and that in which it was injected. Another species difference is involved in the use of such crystallized albumin in man; it is an assumption to apply these results directly to human patients. In so far as the experiments go, however, none of the histologic changes which resulted from the intravenous administration of crystallized bovine albumin can be regarded as a contraindication for the use of the material.

SUMMARY

Rabbits were injected intravenously with crystallized bovine albumin in 25 per cent solution. The dosage was 1 gm. of albumin per Kg. of body weight. Animals were sacrificed at various intervals after one, two, seven and twelve injections.

The injections of crystallized bovine albumin induced no change in state of nutrition or behavior of the rabbits.

The changes in tissues attributed to the albumin were confined to the kidneys, bone marrow, spleen and, to a less degree, to other organs of the reticulo-endothelial system.

Slight enlargement of the spleen was noted grossly. Histologically, there was an increase in the amount of phagocytosis of leukocytes and of red blood cells in the pulp and sinusoids of the spleen. This was

regarded as an accentuation of the process occurring in the spleen of normal rabbits.

Similar sequences of phagocytosis of blood cells were found in the Kupffer cells of the liver, in the lymph nodes and in the bone marrow. In these organs, the process took place to a very slight extent.

In the kidneys there was swelling and dispersion of the cytoplasm of the epithelium in the ascending limb of Henle's loop and in the distal convoluted tubules. These changes were regarded as an indication that some of the albumin was filtered through the glomerulus and entered the tubular epithelial cells more rapidly than it was reabsorbed in the circulating blood.

When injections were repeated, each injection gave rise to another series of tissue sequences which were identical with those resulting from one injection.

The histologic changes were reversible, the organs returning to a normal state even after twelve injections.

Amyloid was not found in any of the rabbits.

Crystallized bovine serum albumin gives rise to somewhat less marked tissue reaction in rabbits than does purified amorphous bovine albumin.

Crystallized bovine serum albumin is not stored in the tissues of rabbits in a form recognizable morphologically. In this respect it differs from methyl cellulose, polyvinyl alcohol and certain other compounds of high molecular weight.

Because of species differences, it is hazardous to assume that the tissue reactions of man to crystallized bovine serum albumin are the same as those of the rabbit. However, no tissue reactions have been found in the rabbit which would contraindicate the use of crystallized bovine serum albumin, were they to occur in human patients.

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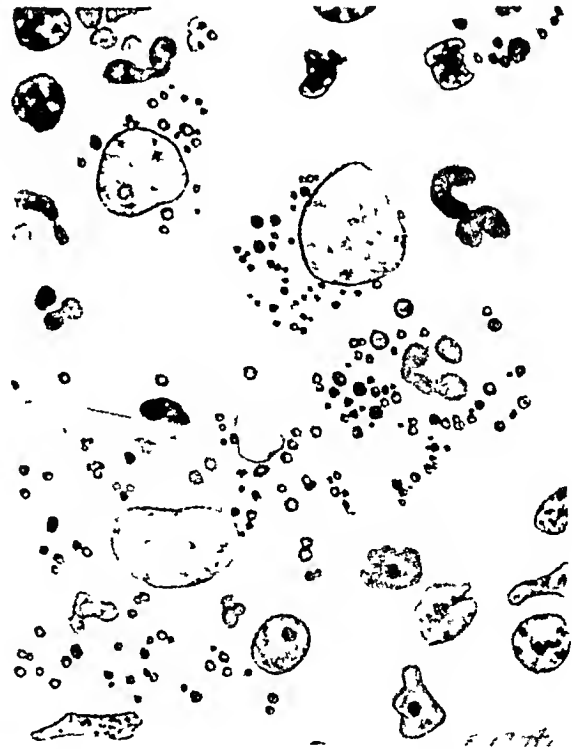
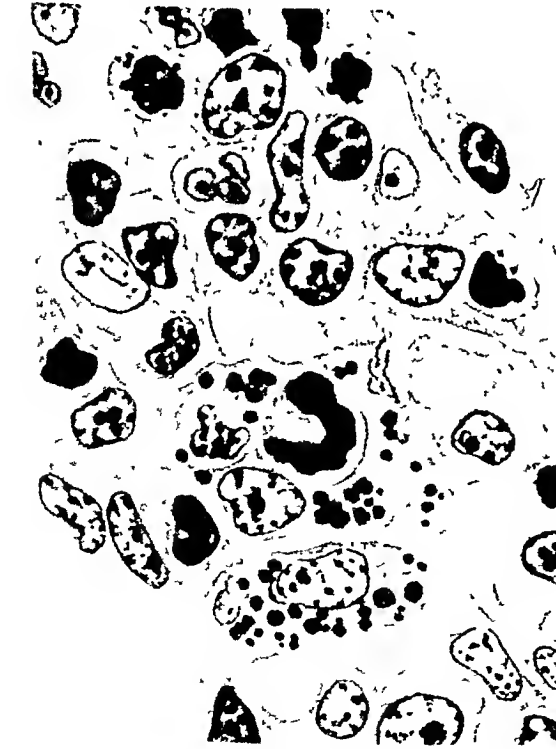
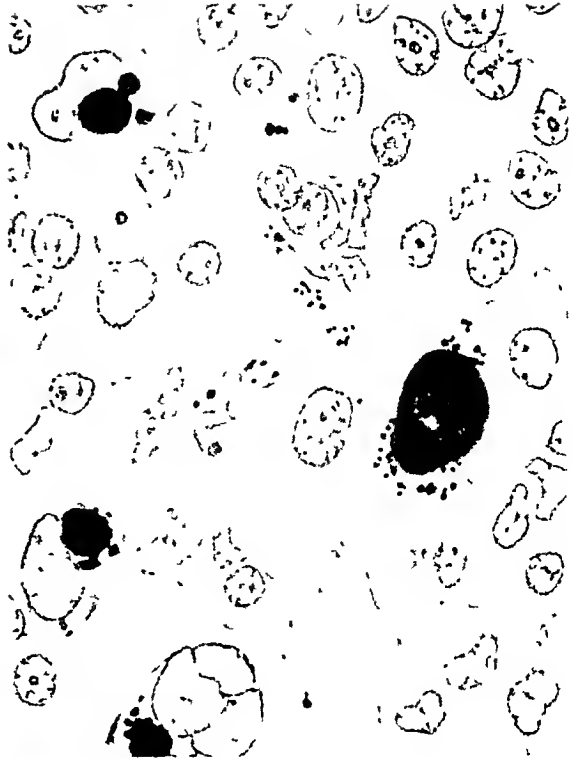
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 33

- FIG. 1. Spleen of control rabbit. Lining a sinusoid and lying free in the pulp there are phagocytes containing fragments of nuclear material. Several erythrocytes are seen in the open meshwork of the pulp. Camera lucida drawing. Phloxine and methylene blue stain. $\times 945$.
- FIG. 2. Spleen of rabbit sacrificed 1 day after the last of seven injections. Several phagocytes contain varying amounts of hemosiderin. In addition, a few red blood cells are seen phagocytosed whole, not yet having been degraded to hemosiderin. Camera lucida drawing. Turnbull's-blue stain for hemosiderin with basic fuchsin counterstain. $\times 660$.
- FIG. 3. Spleen of rabbit sacrificed 4 days after the last of twelve injections. Within the pulp are two large phagocytes laden with fragments of nuclear material. In one of these a phagocytosed polymorphonuclear leukocyte is seen to be undergoing degenerative changes. Camera lucida drawing. Phloxine and methylene blue stain. $\times 945$.
- FIG. 4. Spleen of same rabbit as used for Figure 3. The identity of the nuclear fragments is established by the purple coloration with Feulgen's stain. There are two intact polymorphonuclear leukocytes apparently beginning to undergo degeneration. Camera lucida drawing. Feulgen's stain, Licht Grün counterstain. $\times 945$.



Bailey and Hawn

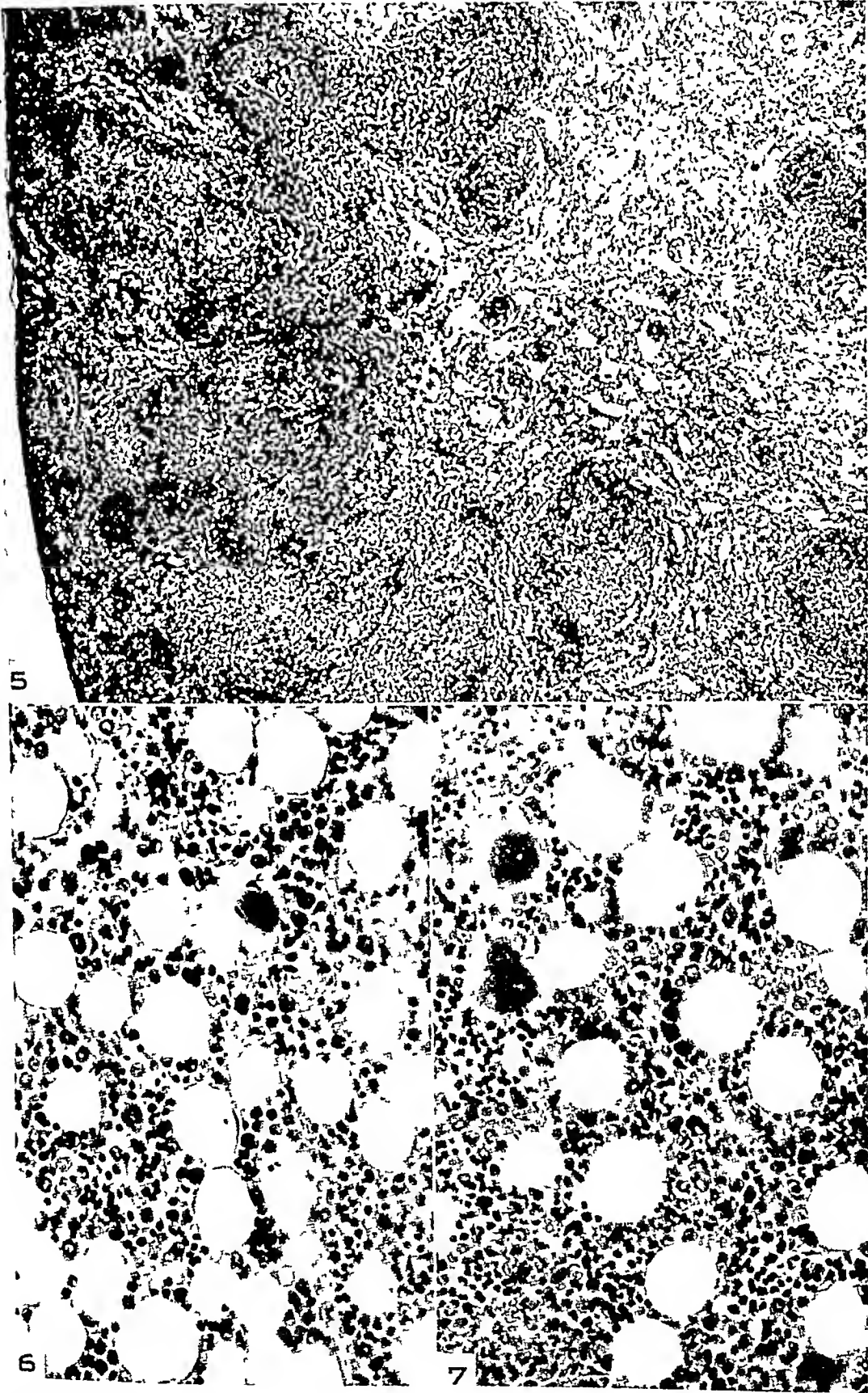
Effect of Crystallized Bovine Serum Albumin

PLATE 34

FIG. 5. Spleen of rabbit sacrificed 5 days after the last of twelve injections. The sequences of degeneration and phagocytosis of erythrocytes and leukocytes have caused no significant alteration in the general architecture. The malpighian corpuscles are moderately prominent and the pulp is somewhat increased in cellularity, particularly beneath the capsule. Phloxine and methylene blue stain. $\times 45$.

FIG. 6. Femoral bone marrow of control rabbit. Phloxine and methylene blue stain. $\times 300$.

FIG. 7. Femoral bone marrow of animal autopsied 5 days following the last of twelve injections. There is slight to moderate increase in the cellularity, with slight increase in the proportion of adult polymorphonuclear leukocytes. General architecture remains normal. Megakaryocytes show no unusual features. This is the most marked degree of hyperplasia in any animal of the series. Phloxine and methylene blue stain. $\times 300$.



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Effect of Crystallized Bovine Serum Albumin

PLATE 35

FIG. 8. Kidney of control rabbit. Phloxine and methylene blue stain. $\times 300$.

FIG. 9. Kidney of rabbit sacrificed 24 hours after the last of seven injections. The distal convoluted tubules, the ascending limbs of Henle's loops and portions of the proximal convoluted tubules show swelling and vacuolation of the lining cells with encroachment upon or obliteration of the lumina. There are no evidences of cell necrosis. Granular protein precipitate is seen in tubular lumina. The glomerulus shows no abnormalities. Phloxine and methylene blue stain. $\times 300$.

FIG. 10. Kidney of rabbit sacrificed 28 days after the last of twelve injections. The tubular epithelium is essentially normal. No scarring, cellular infiltration, or reparative proliferation is evident. The glomerulus is normal in appearance. Phloxine and methylene blue stain. $\times 300$.



THE EFFECT OF POSTURAL HYPERTENSION ON THE DEVELOPMENT OF ATHEROMATOSIS IN RABBITS FED CHOLESTEROL *

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In the course of some experiments originally designed to determine the effects of posture on the distribution of atheromatous lesions in the arteries of rabbits on high cholesterol diets, it was observed that rabbits maintained in an upright position for several hours daily during the feeding period developed more abundant lesions than control animals. There are a number of ways in which the change in posture may produce this result. The metabolism of cholesterol may be changed, the arterial wall may be subject to greater stress and strain and, finally, altered dynamics of blood flow may be implicated. In this report data are presented which indicate that elevation of blood pressure is the chief mechanism involved.

MATERIAL AND METHODS

Six adult rabbits, weighing 2500 gm. each, were placed in upright sitting position for 5-hour periods, 6 days per week. During this time they received 1 gm. of cholesterol 6 days per week. The cholesterol was dissolved in 6 cc. of hot olive oil and made into a paste with bran according to the method of Harrison.¹ This diet was supplemented with commercial rabbit chow and fresh vegetables. Two rabbits were sacrificed after 4 weeks, two after 8 weeks and two after 12 weeks.

Two adult rabbits, weighing 2500 gm. each, were maintained in upright sitting position for 5-hour periods 6 days a week for 12 weeks, but were not fed cholesterol.

Seven adult rabbits, weighing 2500 gm. each, were fed 6 days a week 1 gm. of cholesterol dissolved in 6 cc. of hot olive oil absorbed in bran flakes. These were kept under usual laboratory conditions in individual standard rabbit cages. Two were sacrificed after 8 weeks, four after 12 weeks and one after 17 weeks.

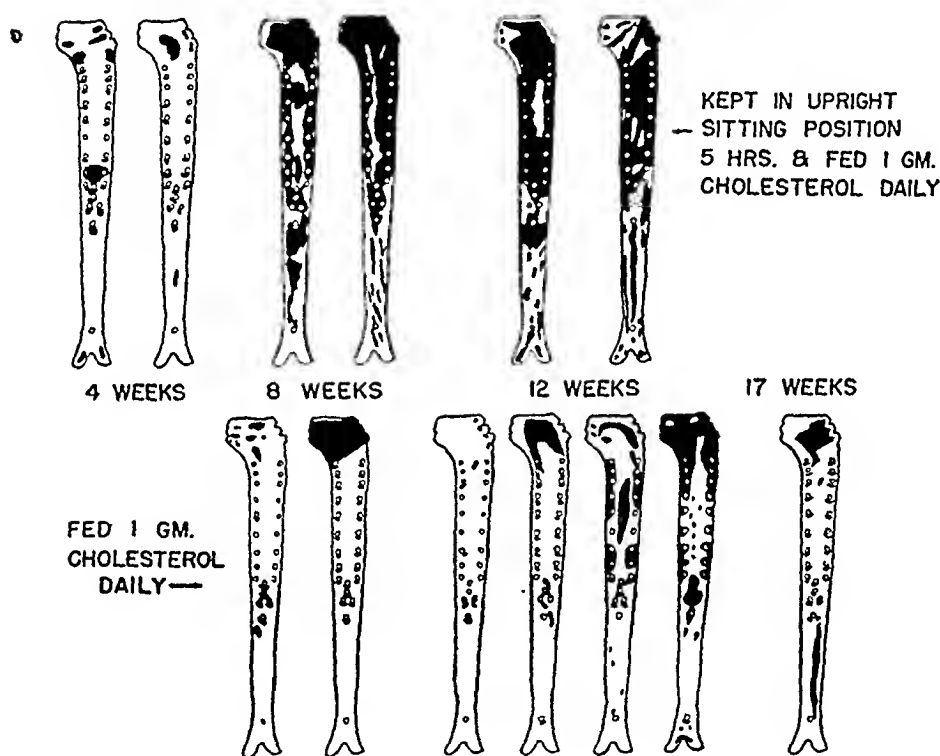
The rabbits were maintained in the upright position by placing them in cylindrical glass jars 32 cm. high and with an internal diameter of 14 cm. The bottoms of the jars were covered with a layer of sawdust about 3 cm. deep. The animals fitted into these jars rather snugly but without discomfort, their haunches resting on the sawdust layer and their heads protruding above the tops of the jars. They seldom struggled to escape and usually rested quite peacefully during the

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whole procedure. Occasionally after several weeks the rabbits learned to escape from the jars. Coarse chicken wire baskets, cylindrical in shape and 14 cm. high, were then fitted over the tops of the jars. The procedure seemed to have no deleterious effect on the general health of the animals. They ate well and actually gained weight.

At the time of sacrifice the aorta was stained *in toto* with Sudan IV and the size and distribution of intimal plaques mapped out diagram-

THE DISTRIBUTION OF INTIMAL LIPID IN THE AORTAS OF RABBITS KEPT IN UPRIGHT SITTING POSITIONS WHILE ON HIGH CHOLESTEROL DIETS



Text-Fig. 1. The distribution of intimal lipids in the aortas of rabbits kept in upright sitting position while on high cholesterol diets. The shaded areas indicate lipid deposits. The aortas in the lower row are from rabbits fed with cholesterol but not subjected to postural change.

matically (Text-Fig. 1). The aortas were then fixed in Kaiserling I solution and photographed (Fig. 1). Subsequently they were washed in running water for 72 hours and placed in Zenker's solution. The entire vessel was rolled into a coil, embedded in paraffin, and representative sections were stained with hematoxylin and eosin, hematoxylin and Weigert's elastic tissue stain, Weigert's elastic tissue stain and van Gieson's stain, and by the Foot-Bielchowsky methods.

RESULTS

The two animals sacrificed after 4 weeks of cholesterol feeding and daily periods in the vertical position showed macroscopic evidence of lipid deposition in the aorta. Previous experience with this feeding method has shown that after 4 weeks there are seldom any grossly visible plaques, although lipid-containing cells in small aggregates are usually demonstrable in the intima microscopically. The amount present in these two aortas was therefore abnormally great. The position of the lipid masses was not strikingly unusual, most of it being at the margins of branch orifices. Rather more lipid was noted in the lower thoracic portion and about the orifices of large abdominal branches and rather less in the ascending aorta than is usually the case.

After 8 weeks the four control animals showed intimal plaques of varying size and number. The largest ones were found in the ascending part of the aorta and the arch. In every instance large areas of the intima were free of lipid deposits. Both rabbits which were maintained in the upright position during 8 weeks of cholesterol feeding showed intimal plaques which were continuous or confluent over almost the entire aorta, so that the small lipid-free zones were entirely enclosed by lipid-containing areas which isolated them from each other. The plaques were abundant in the abdominal portion of the vessel although somewhat less so than in the proximal regions. The impression was thus gained that not only were lipid deposits greatly increased by the change in posture but that there was a tendency for them to appear in unusual locations. However, their presence in the abdominal aorta might be considered to be simply an expression of the greater total amount of fat deposited rather than a real reversal of the usual pattern observed in control animals.

After 12 weeks there was still a striking difference between the two animals which were fed cholesterol and maintained in the upright position and the two animals which were fed cholesterol but allowed normal posture. One animal fed cholesterol continuously for 17 weeks without being placed upright showed less atheroma than either of the two rabbits kept upright daily for 8 weeks.

In summary, rabbits maintained in upright sitting position in glass jars for 5 hours daily during cholesterol feeding developed larger intimal lesions than control animals fed with equivalent amounts of cholesterol. Furthermore, the plaques developed more quickly and tended to be more widely distributed in the aorta in the animals which were kept upright.

Three possibilities suggest themselves to account for these findings.

(1) Rabbits kept in the upright sitting position may develop hypercholesterolemia more rapidly than the controls. (2) The mechanical effects of abnormal posture may injure the aortic wall in such a fashion as to make it more vulnerable to lipid deposit. (3) The blood pressure may be increased during the period of postural change and the observed acceleration of lipid deposit may be due to relative hypertension. Each of these possibilities was investigated.

Cholesterol Content of Blood in Rabbits Maintained in Upright Sitting Position

Man and Peters ² have shown that when the human subject is kept in the erect position and at rest for periods of 30 minutes there is a relative increase of plasma protein and cholesterol of over 10 per cent. This they attributed to an increase in tissue fluid at the expense of blood volume, the larger relatively impermeable molecules such as protein and cholesterol failing to pass through the capillary membranes and being retained in the circulation. Such a phenomenon, if present in rabbits kept in upright sitting position, might account for transient periods of elevated blood cholesterol and thus explain the increased tendency for atheromatous lesions to develop. Five rabbits were tested. Five cc. of blood were removed from ear veins just before the rabbits were placed upright in jars and again at the end of 5 hours. The blood obtained was analyzed for protein content by the specific gravity drop method of Drew, Scudder and Papps ³ and for cholesterol by the Schoenheimer-Sperry technic.⁴ Four of the five rabbits were then fed 1 gm. of cholesterol in 6 cc. of olive oil daily. The test was repeated after 4 and 8 weeks. To control the factor of dilution of blood by bleeding, at the final test the first blood samples were taken at the termination of the upright sitting period and the second samples 15 hours later. The results are listed in Table I. They failed to show any consistent change in either protein or cholesterol levels which can be attributed to postural change. The level of blood cholesterol attained in the four rabbits which were fed with this material showed such variation both after 4 and 8 weeks that it is not possible to say whether these values are greater than those which might be observed in cholesterol-fed rabbits not subjected to changes in posture. Weinhouse and Hirsch ⁵ reported serum cholesterol values, not unlike those recorded here, in their rabbits which were fed approximately the same amounts of cholesterol. The amount of cholesterol deposited in extravascular sites did not differ appreciably between the rabbits kept in erect position and the controls. In fact the amount of lipid found in the aortic intima and other sites did not correspond closely to the

blood cholesterol content. It may be concluded that the observed increase in aortic lipid deposit in rabbits maintained in erect sitting position during cholesterol feeding periods is not dependent on a tendency toward hypercholesterolemia.

TABLE I

Serum Cholesterol and Protein in Rabbits Before and After Being Placed in Upright Sitting Position for 5 Hours

Rabbit no.	Position	Period of cholesterol feeding					
		None		4 weeks		8 weeks	
		Total cholesterol	Protein	Total cholesterol	Protein	Total cholesterol	Protein
		(mg. per 100 cc.)	(gm. per 100 cc.)	(mg. per 100 cc.)	(gm. per 100 cc.)	(mg. per 100 cc.)	(gm. per 100 cc.)
1	Prone	66	6.83	290	5.81	...	5.37
	Upright	65	6.02	280	5.99	600	5.17
2	Prone	59	5.17	801	5.24	1430	5.27
	Upright	60	6.21	782	5.62	1430	4.99
3	Prone	61	5.69	460	5.27	625	5.85
	Upright	59	4.86	482	5.89	603	5.78
4	Prone	67	7.07	395	5.95	520	6.29
	Upright	64	6.05	385	5.48	530	5.58
5 Not fed cholesterol	Prone	64	6.46	63	6.43	58	6.50
	Upright	63	6.86	59	5.78	59	5.92

The Mechanical Effect on the Aorta of Maintaining Rabbits in Upright Sitting Position

Klotz⁶ demonstrated that by suspending rabbits by their hind legs, he could accelerate the development of sclerotic changes in the aorta. The lesions produced were not unlike those produced by adrenalin injections and sometimes observed spontaneously. It has been shown (Anitschkow⁷ and Harrison¹) that the presence of medial defects in the rabbit's aorta will influence the deposition of intimal lipid following cholesterol feeding. In fact, Anitschkow⁸ combined cholesterol feeding with suspension of the rabbits by their hind quarters but failed to observe any marked acceleration of lipid deposition.

The procedure used in these experiments is much less drastic than that employed by Klotz.⁶ Nevertheless the aortas were studied histologically for evidence of structural change which might be attributed to the postural treatment alone. The aortas of two control rabbits which were kept erect but were not fed with cholesterol were available in addition to those of the other animals. Areas of medial fibrosis or scarring were occasionally observed but these were no more extensive

than in the control animals not subjected to postural change. There is, therefore, no morphologic evidence that mechanical trauma to the aorta is a factor in the increased deposition of lipid.

The Effect on the Blood Pressure of Maintaining Rabbits in Upright Sitting Position

Intra-arterial pressure is generally believed to be a factor in the development of human atheromatous lesions. Such lesions are found in parts of the vascular system where the pressure is normally relatively high and seldom in very small systemic arteries, pulmonary arteries, or veins. There is frequent association of hypertension and advanced arteriosclerosis. When conditions in the pulmonary circulation lead to increased intra-arterial pressure, intimal lipid-containing plaques frequently develop in this unusual site. In fact, some authors assign a primary rôle to the influence of hypertension on the development of arteriosclerosis. However, many subjects exhibit advanced lesions without clinical evidence of hypertension or anatomic evidence of cardiac hypertrophy. Another more striking discrepancy is the fact that individuals with "malignant" hypertension with the pressure markedly elevated for many years may die in renal insufficiency and, although the arteriolar bed may show striking changes, the larger arteries frequently present surprisingly few intimal lesions. Thus there is evidence to indicate that the presence of hypertension is a factor favoring the development of arteriosclerosis, but there is also good reason to believe that other conditions must likewise be fulfilled.

The rôle of the blood pressure in the development of the experimental lesions in cholesterol-fed rabbits is less clear. Anitschkow⁸ claimed to have accelerated the development of these lesions by producing hypertension experimentally. He placed constricting ligatures on the abdominal aorta just above the bifurcation before cholesterol feeding was begun. However, it is not likely, according to the more recent findings of Goldblatt, Kahn and Hanzal,⁹ that a ligature placed at a point so far beyond the renal orifices will result in sustained hypertension. It has been claimed that the feeding of cholesterol alone produces an elevation of blood pressure.¹⁰ However, there are no convincing blood pressure measurements to prove this. Katz, Sanders, Megibow and Carlen¹¹ found normal blood pressures in rabbits on cholesterol diets even though the heart weights were increased at the time the animals were sacrificed. In a recent publication, Dill and Isenhour¹² reported that 7 of 12 rabbits with experimental renal hypertension of long duration developed aortic atheromatous plaques.

These rabbits were fed stock diets and failed to show elevation of blood cholesterol.

The pulmonary arteries in cholesterol-fed rabbits are almost as commonly the seat of lipid deposits as are the systemic arteries. This suggests that the blood pressure is of less significance in the pathogenesis of rabbit lesions than it is in human lesions.

Nevertheless, in order to determine if this might be a factor in the accelerated production of intimal lesions the blood pressure of ten normal and of five already hypertensive rabbits was measured indirectly by the ear capsule method of Grant and Rothschild¹³ in the prone and subsequently in the upright sitting position. This method has been shown to give consistent results although the values obtained from the medial artery of the ear are considerably lower than those by direct cannulation of the carotid artery. The hypertensive rabbits had had one kidney wrapped in cellophane and the opposite one subsequently removed. The procedure has been shown by Page¹⁴ and by Graef and Page¹⁵ to be an effective method of producing hypertension in dogs.

TABLE II

The Effect of Posture on the Systolic Blood Pressure in Normal Rabbits

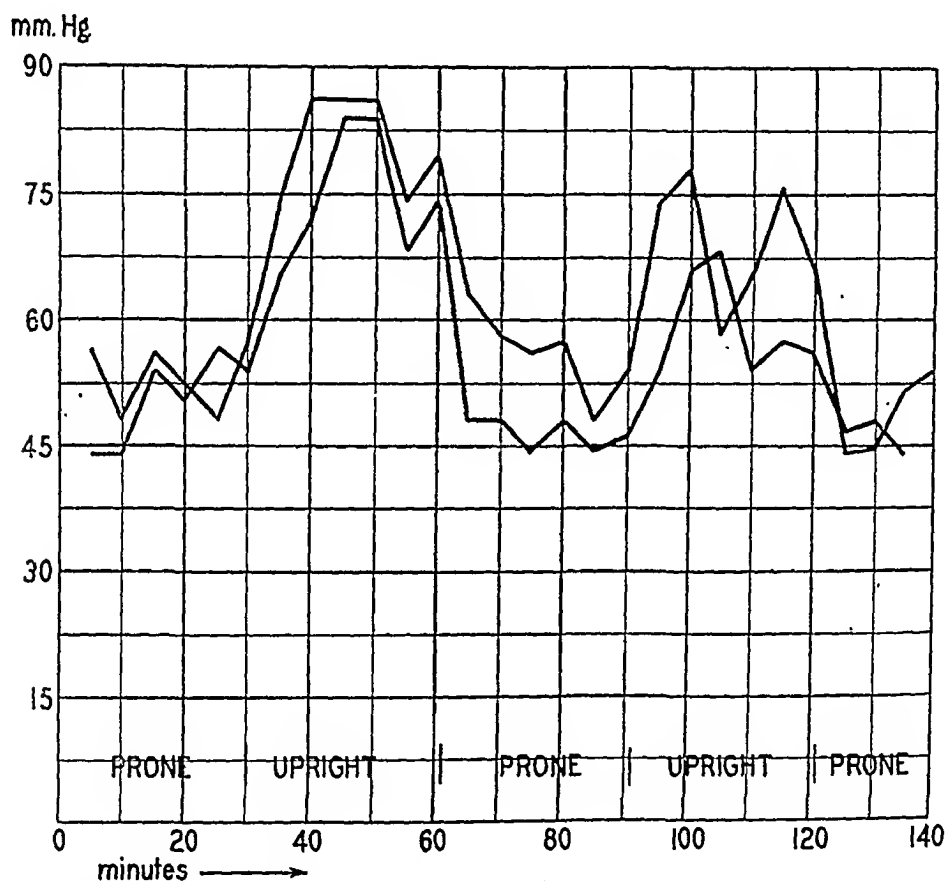
Rabbit no	Position		Per cent increase
	Prone	Upright sitting	
1	43 mm. Hg	70 mm. Hg	62.8
2	45 mm. Hg	68 mm. Hg	51.1
3	53 mm. Hg	80 mm. Hg	50.9
4	56 mm. Hg	70 mm. Hg	25.0
5	51 mm. Hg	75 mm. Hg	47.1
6	46 mm. Hg	59 mm. Hg	28.3
7	77 mm. Hg	87 mm. Hg	13.0
8	73 mm. Hg	94 mm. Hg	28.8
9	61 mm. Hg	97 mm. Hg	59.0
10	84 mm. Hg	105 mm. Hg	25.0
Mean	59 mm. Hg	81 mm. Hg	39.1

TABLE III

The Effect of Posture on the Systolic Blood Pressure in Hypertensive Rabbits

Rabbit no.	Position		Per cent increase
	Prone	Upright sitting	
1	85 mm. Hg	130 mm. Hg	52.9
2	99 mm. Hg	124 mm. Hg	25.3
3	117 mm. Hg	165 mm. Hg	41.0
4	122 mm. Hg	149 mm. Hg	22.1
5	115 mm. Hg	125 mm. Hg	8.7
Mean	108 mm. Hg	139 mm. Hg	30.0

Both normal and hypertensive rabbits showed a consistent elevation of systolic blood pressure when placed upright in glass jars. This elevation averaged 39.1 per cent in the case of normal rabbits (Table II) and 30.0 per cent in those that were already hypertensive (Table III). The elevation was sustained as long as the rabbit was kept in the upright position and it could be reinduced intermittently (Text-Fig. 2). No significant change in heart weight was observed, when the animals were sacrificed, in the rabbits kept in upright position



Text-Fig. 2. Systolic blood pressure in two rabbits kept alternately in prone and upright sitting positions.

as compared with the controls. Therefore it cannot be claimed that the hypertension produced was of sufficient duration or intensity to lead to cardiac muscular hypertrophy.

The exact mechanism by which the blood pressure is altered by the procedure employed is not obvious. In addition to the altered dynamics of circulation introduced by the abnormal position there are other factors which must be considered. These include excitement, retention of body heat in the glass container and external pressure to the soft abdominal parts with its secondary effects on respiratory

movements. All of these may have participated in some measure in producing the rise in blood pressure. Simple mechanical elevation alone is evidently insufficient to produce sustained hypertension. The blood pressure of anesthetized rabbits, fastened by their extremities on animal boards, was measured by direct cannulation of the carotid arteries. When the board was tilted at an angle of 75 degrees, elevating the head of the rabbit, only a slight transient rise of pressure was usually elicited; the rise persisted for a few minutes and was followed by a fall, frequently to below the original level.

The initial excitement attendant on manipulating the rabbits into the jars probably did not play an important rôle as the rise in pressure persisted during the entire period of postural elevation. During most of this period the rabbits were at rest or even asleep. Increased retention of body heat was a more conspicuous feature. The glass jars became quite warm. The ears showed evidence of increased circulation; they usually became very warm and both arteries and veins stood out very prominently on transillumination. However, change in body temperature alone cannot be held accountable for the observed increase in pressure. Rabbits kept in the glass jars in a prone position showed no elevation of pressure. Moreover the rapid rise that followed when the jars were subsequently placed vertically indicated that the change in posture was the decisive factor.

DISCUSSION

The only demonstrable difference between animals kept in an upright sitting position and the controls was in the level of blood pressure. Since high blood pressure is recognized as a predisposing factor in the development of intimal plaques in man, one is led to offer this as an explanation for the increased deposition of intimal lipids. As already pointed out, in cholesterol-fed rabbits the blood pressure is of less obvious significance in the production of intimal deposits. The marked retention of this material in the body fluids is generally considered to be the chief cause of its eventual penetration into arterial walls. Even under conditions of normal pressure in the systemic arteries and in the pulmonary circulation where the intra-arterial tension is considerably lower, lipid deposits occur with cholesterol retention. However, the blood pressure must also be of importance, even in the rabbit, since the veins and very small arteries are seldom involved. There is thus good reason for believing that in hypertensive rabbits the atheromatous process is intensified. The limited degree of elevation of pressure may cast some doubt on the validity of this suggestion, but no other plausible theory can be advanced. If it is true, the experimental

rabbit lesion has one more feature in common with the spontaneously occurring human disease, namely, it is influenced by blood pressure levels.

Because of the obvious difference in the way the rabbit metabolizes ingested cholesterol as compared to man, it has been seriously questioned whether the arterial lesions thus produced have any relation to human arteriosclerosis. This objection is undoubtedly sound since the underlying mechanism which causes marked retention of this material in the tissues and fluids of the rabbit need not obtain in man. Nevertheless, this does not exclude the possibility that the pathogenesis of the arterial lesions themselves may be the same in both species. The histologic resemblance of the lesions in the rabbit to early human lesions is striking. The tendency of the lesions to be localized at the orifices of arterial branches is common to both species. If, as the data presented indicate, high blood pressure promotes the development of the lesions in rabbits, then the likelihood that they are analogous to the human lesions is further increased.

The findings reported here do not shed much light on the manner in which hypertension may accelerate intimal deposition of lipids. The prevalent explanation is that high pressure injures the artery at especially vulnerable points and that injury favors the deposit of lipids. The acute nature of the process in these experiments rules out injuries of the type that are slow or gradual in development. In a previous report an alternative explanation has been offered for the focal character of lipid deposits in arteries, namely, the involved areas represent parts of relative fixation or immobility to which lipids converge from the adjacent intimal tissue. According to this view, the influence of hypertension would be to increase the rate of penetration of lipids into the intima as a whole. The rapid effect of even moderate degrees of hypertension in the rabbit would support this latter interpretation.

The observations of Dill and Isenhour¹² establish an even closer relationship between hypertension and the formation of atheromatous plaques in the aortas of rabbits. Apparently, even without supplementing the diet with cholesterol, lipid deposits in the intima frequently form in hypertensive rabbits. These lesions were not observed, however, when the hypertensive state had lasted less than 6 months. When present, they were for the most part quite small and limited to the arch of the aorta or to the orifices of the intercostal arteries. There is little doubt that in the experiments reported here, the cholesterol feeding played a decisive part in the rapid development of large confluent lipid deposits.

SUMMARY

The systolic blood pressure of rabbits placed in upright sitting position in glass jars is significantly elevated. This elevation persists during the entire period of postural change, but the blood pressure returns to normal when the rabbits are removed from the jars. This elevation of pressure occurs not only in normal rabbits but in those previously rendered hypertensive by unilateral cellophane perinephritis followed by contralateral nephrectomy.

Rabbits fed high cholesterol diets and placed in the upright sitting position for 5 hours daily develop more abundant intimal deposits of lipids in their aortas than rabbits fed equal amounts of cholesterol but not subjected to postural change. The intimal lesions develop more quickly, are larger and tend to be more widely distributed over the entire aorta, including the abdominal portion.

It seems reasonable to conclude that even moderate degrees of elevation of blood pressure facilitate the passage and retention of lipids in the aortas of cholesterol-fed rabbits.

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DESCRIPTION OF PLATE

PLATE 36

FIG. 1. Photograph of rabbits' aortas showing distribution of intimal plaques. The lipid is stained with Sudan IV and photographs dark gray in contrast to the lighter lipid-free areas. The two aortas on the left are from rabbits fed cholesterol for 8 weeks and maintained in upright sitting position for 5 hours daily. The two on the right represent the maximum and minimum deposits seen in rabbits fed cholesterol for 12 weeks but not subjected to postural change. There are deposits of greater extent in both thoracic and abdominal portions of the two aortas on the left.



Wilens

Postural Hypertension and Atheromatosis

1926

INTERSTITIAL CELL GROWTHS OF THE TESTICLE *

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The differentiation between hyperplasia and neoplasia in respect to certain rare tumors such as those of interstitial cells is difficult chiefly because of lack of familiarity with the variations of the normal cells. We report two cases of hyperplasia of interstitial cells and two cases of local tumor of interstitial cells. The criteria for the classification of these cases are based on examination of a series of 370 other testes in our files. Since the purpose was to obtain a working knowledge of the variations in the number and distribution of interstitial cells, special sections were not made. Each testis was graded for the following items: number of interstitial cells, atrophy, spermatogenesis. The number of interstitial cells was marked as one to four plus. Four plus was considered high normal or questionable hyperplasia. Tubular atrophy was graded similarly. One plus indicated slight atrophy of tubules; four plus, complete atrophy. Early thickening of the basement membrane without other evidence of atrophy was noted as such rather than as atrophy. The degree of spermatogenesis was indicated as normal, slightly or markedly diminished, and absent. The amounts of fibrosis of stroma, inflammatory reaction, or vascular disease were described. We did not study the interstitial cells for histologic variations except to note whether or not they corresponded to the so-called normal. Unusual distribution of the cells was noted. Marked local variations were described rather than graded, as, for example, spotty complete atrophy in an otherwise normal testis. From the statistical point of view, the data were inconclusive. One to three plus interstitial cells were about evenly distributed among the testes with one to four plus tubular atrophy; of 16 testes with four plus atrophy, 4 had three plus interstitial cells and 2 had four plus interstitial cells. Of the testes without spermatogenesis, there were more with few interstitial cells than otherwise.

An abnormality in the number of interstitial cells was classified as: (1) hyperplasia—an increase of interstitial cells between the tubules without destruction or displacement of the tubules beyond the limits of the tumor; (2) local tumor—a discrete nodule or group of interstitial cells locally replacing or displacing the seminiferous tubules; (3) malignant tumor—increase of interstitial cells with anaplasia, destruction of tubules and metastases.

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THE INTERSTITIAL CELLS IN THE NORMAL TESTIS

The interstitial cells are arranged in compact groups in spaces between the tubules⁴² without a consistent relationship to other structures. They are usually polyhedral, sometimes with short processes, and they vary from 14 to 21 μ in diameter. The nucleus is relatively large and spherical, often wrinkled, contains one or two large nucleoli and numerous coarse granules. Binucleate cells are not uncommon⁴² and multinucleate cells with as many as thirty nuclei have been described.⁴³ The two striking characteristics of the cytoplasm are brown granules, thought to be waste pigment (lipofuscin), and crystalloids (of Reinecke). The latter are rod-shaped bodies of unknown composition with rounded or pointed ends,⁴² which are polychromatic, staining red with safranin, violet by the method of Bizzozero, red or green by Benda's method.²² They are not always present, but when numerous or large are quite striking.⁴² Less conspicuous constituents of the cytoplasm are the mitochondria and highly refractile granules, some of which are neutral fats and lipoids. Suitable technics bring out an attraction sphere with round or rod-shaped centrioles near the nucleus and Golgi apparatus around the sphere.⁴²

The number of interstitial cells varies with age. They are few during intrauterine life and in the first postnatal year.^{44, 57} The density of the interstitial tissue, including the interstitial cells whose appearance is unlike that of the adult,⁷⁰ decreases as the growth of the tubules increases at the seventh month in utero.^{18, 33} The cells remain relatively few in number until puberty, when they are more numerous.²⁸ From puberty to maturity, there is an increase in the number of interstitial cells.

There is no uniformity among different species of animals as to the number of interstitial cells, their time of appearance, and cycles of growth,^{2, 10, 33, 66} although in cats the development of interstitial cells is similar to that in man.³⁶

In the normal adult testis, the number of cells has not been studied with the same care as the morphology. Few actual counts have been made and most of the data are based either on general impressions or on calculations.^{30, 60} Sand and Okkels^{61, 62} have pointed out that the possible limits of normal in regard to the number of interstitial cells surpass what is generally described as the normal histology. They studied testes from 39 castrated sexual offenders and from 33 individuals between the ages of 20 and 70 years who had died suddenly because of accident or suicide. Only 8 of the 33 men in the second group had so-called normal testes. Teem⁷⁴ found the individual variation greater in middle age than in young persons. He also noted a

tendency toward clumping of cells after middle life. In our group of 279 testes from persons of known ages, associated with a wide variety of lesions, 63.5 per cent had a normal number and distribution of interstitial cells. In 29.2 per cent the cells were either absent or extremely rare and in 7.3 per cent they were very numerous, possibly in some instances abnormal.

THE RELATIONSHIP OF INTERSTITIAL CELLS TO HORMONAL INFLUENCES

The development of tubules and interstitial cells in inverse ratio at different periods of intrauterine life in man has been linked with the concentration of anterior pituitary-like hormone in maternal and fetal blood. Thus the active growth of the tubules as compared with the interstitial cells at the seventh month of gestation is accompanied by an increase in maternal anterior pituitary-like hormone, and the decrease in this substance by 50 per cent and 25 per cent in maternal and fetal blood, respectively, at the ninth month is associated with the reverse relations of tubules and interstitial cells.^{18, 33} Hyperplasia and local tumor of the interstitial cells have been associated with developmental abnormalities of the genitalia, precocious puberty, and tumors of the testis and pineal gland (Tables I and II).

There is much information relative to the interrelationship of interstitial cell growth and activity and certain internal secretions. The most obvious fact in this connection is the well-known seasonal variation in interstitial cells in certain animals. Different interpretations can be drawn from the experiments concerning the effect of anterior pituitary hormone on interstitial tissue.^{11, 20, 23} Prolonged injection of prolan for an unstated period in infant and adult mice and rats has been shown to increase the number of interstitial cells, especially in the region of the rete, while it caused only slight atrophy of the tubules.³⁵ It was not determined whether prolan A or B was responsible. In the mouse and rat, the anterior pituitary stimulates proliferation of interstitial cells.¹¹ Hyperplasia with some tendency toward neoplasia has been found about 2 months after injection of 3 mg. doses of triphenylethylene in Strong A strain of mice, 4 to 7 weeks old.⁶ The estrogen was administered weekly. The neoplastic cells were characterized by very acidophilic cytoplasm, large nuclei and nucleoli, and mitotic figures. An interesting finding was focal hematopoiesis among the tumor cells. In one mouse treated for 52 weeks, the new growth extended through the capsule into the connective tissue of the spermatic cord and metastasized to the upper pole of the right kidney. An interstitial cell tumor was produced in

TABLE I
Interstitial Cell Hyperplasias

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structures	Metastases	Mitoses	Remarks
Stroebe ¹³	1897	Hyperplasia; pseudohermaphroditism masculinus in testis	63	"Bean-shaped"		Autopsy	Bilateral	Groups; bands; macroscopically visible islands surrounded tubules				Testicles on either side of elongated uterus with well developed round ligaments and tubes
Pick ¹⁴	1905	Hyperplasia; internal masculine pseudohermaphrodite	38	3.5 x 2 cm.	Multiple; largest, 1 mm. in diameter			Groups; strands, nodules; diffuse sheets overgrowing the tubules; tubules embedded in tumor; largest mass 1 mm. in diameter				
Dürck ¹⁵ (Case 1)	1907	Hyperplasia	25	1.8 x 1 cm.; 7 gm. with epididymis		Autopsy	Bilateral	Sheets of cells between atrophic tubules				
Dürck ¹⁶ (Case 2)	1907	Hyperplasia	64	Hazelnut-sized		Autopsy	Bilateral	Cells, large in size, increased between the tubules				Testes in peritoneal cavity; pattern suggestive of polymorphous pigment cell sarcoma
Dürck ¹⁶ (Case 3)	1907	Hyperplasia	45	Small		Autopsy	Bilateral	Intertubular masses				
Dürck ¹⁶ (Case 4)	1907	Hyperplasia	43	Right: larger than walnut size; left: small		Autopsy	Unilateral (right)	Seminiferous tubules had almost entirely disappeared; cell-poor bridges of fibrillary tissue with intervening spaces entirely filled by polygonal cells with vesicular nuclei			Atypical	Resembled sarcoma; left testicle: diffuse fibrous orchitis without interstitial cells

TABLE I (Continued)
Interstitial Cell Hyperplasias

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structures	Metastases	Mitoses	Remarks
Orsós ⁴⁶	1931	Hyperplasia in pseudo-hermaphrodite female	44	Nut-sized		Surgery	Bilateral	Increased interstitial cells between the tubules which are partially hyalinized; a few degenerated spermatogens present; many cells smaller than normal; adenomatous pattern in same				Testicles in hernial sac; epididymis present as well as cord, latter ending blindly and continuing as cord leading to vagina (blind); uterus and adnexa present
Grynfeldt, Truc and Guibert ⁴⁷	1933	Hyperplasia	56			Surgery	Unilateral	Cords; masses compressing tubules, perivascularly	(1) Walls of tubules; (2) center of fibrous cord replacing seminiferous canal	None	(Amiotoses)	Testicle 2 cm. above pubis
Le Chuiton, Prade, Berge and Pennan- each ⁴⁸	1935	Adenoma	43			Autopsy	Unilateral	Seminoma with hyperplasia of interstitial cells; seen between the tubules and among the seminoma cells; interstitial cells are glycogen-free in contrast to seminoma cells	None	None	Frequent	At site of seminoma-metastases there were no interstitial cell metastases; opposite testicle, normal; no adenoma of interstitial cells present
Cleghorn, Hyland, Mills and Linell ⁴⁹	1938	Hyperplasia of interstitial cells; pincaloma	23	Atrophic		Autopsy	Bilateral	Complete atrophy of tubules; hyperplasia of interstitial cells				Pincaloma with invasion of brain stem and hypothalamic region; internal hydrocephalus
Warren and Olshausen	1942	Hyperplasia	46	6.5 x 5.0 x 2.5 cm.	Same as testis	Surgery	Bilateral	Closely packed cells between the tubules	None	None	None	X-radiation postoperatively; urine after operation positive for testosterone and negative for estrin
				4.0 x 3.0 x 2.5 cm.	Same as testis	Surgery		Moderate number of cells between the tubules; in sheets in the epididymis	Epididymis	None	Occasional	This testis removed 3 mos. after the other; hyperplasia in testis, greatest near rete
Warren and Olshausen	1942	Hyperplasia	51	3.5 x 2.0 x 1.8 cm.	Same as testis	Surgery	Bilateral	Reddish brown; increase of cells between tubules	None	None	None	

TABLE II
Interstitial Cell Tumors

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structures	Metastases	Mitoses	Remarks
Chevassu ¹²	1906	Tumor	27	Small hen's egg	Replaced entire testicle	Surgery	Unilateral	Lobules separated by connective tissue strands; histologically benign; tumor limited by fibrous shell containing a few degenerated tubules	None	None		Alive 22 months post-operatively; ectopic
Kaufmann ¹¹ (Case 1)	1907	Tumor	30	(a) 210 gm., 9 cm. long, 1 cm. in diameter; (b) 140+ gm., 9 cm. long	Entire testicle excepting subalbugineal area	Surgery	Bilateral	Lobules separated by connective tissue; seminiferous tubules only beneath tunica albuginea; largest lobule is cherrystone-sized	None			See next case
Kaufmann ¹¹ (Case 2)	1908	Tumor	34	150+ gm., 9 cm. long		Surgery	Unilateral	Similar to above	None			Other testicle, left <i>in situ</i> , was voluminous; author considered all three (including previous case) probably benign, although polymorphism of cells was considered suggestive of malignancy
Rowlands and Nicholson ¹⁸	1929	Adenoma	9	5 x 4 x 3.5 cm. (5 x right testicle)	Almost entire testicle	Surgery	Unilateral	Cords and solid sheets; capsule			None	Precocious puberty (onset stage, 6 to 7 years); some regression of pubertal signs 1 year later (McGeer information)
Slanina ¹⁸	1930	Interstitial cell tumor	45		Hen's egg; 10 cm. long, 94 gm.	Surgery	Unilateral	Round cells; numerous vacuoles in protoplasm				
Pana ²⁰	1931	Interstitialoma	16	Same as tumor	28 gm., 6.2 x 3.1 x 2.9 cm. (includes tumor, connective tissue and periphery)	Autopsy	Unilateral	Uniform mass with some connective tissue strands; tubules at periphery with connective tissue				Right testicle, 3.5 gm.; small tubules; interstitial cells abundant with some small nodules
Stewart, Bell and Roehlke ¹¹	1936	Interstitial cell tumor	5	Maximum transverse diameter, 2.8 cm.	1.8 x 1.5 x 1 cm.	Surgery	Unilateral	Not sharply demarcated; cords and solid sheets				Precocious puberty at 4 years with small tumor, 1 cm. in diameter, in testicle; pituitary, pineal, adrenal reported normal; large testicle considered due to tubules rather than to tumor

TABLE II (Continued)
Interstitial Cell Tumors

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structures	Metastases	Mitoses	Remarks
Jemerin ¹⁸	1937	Interstitial cell tumor	35	6.5 x 6 x 3.5 cm.	Entire testicle except small area at lower pole	Surgery	Unilateral	Anastomosing strands, nests, or broad sheets	None		None	Area at pole contained atrophic tubules without interstitial cells
Budd ⁹ and Hunt and Budd ¹⁷	1937 1939	Interstitial cell tumor	42	5 x 3.5 x 3 cm.	2 x 2 x 2.5 cm.	Surgery	Unilateral	Elongated or anastomosing columns; capsule penetrated in some places		None		Tubules outside capsule were atrophic; gynecomastia said to regress following removal of testicle; urine and tumor negative for prolan; 20 mouse units of estrin in 1.73 gm. of tumor; positive Zondek reaction equivalent to 1000 units of luteinizing hormone per liter of urine
Braun ⁷	1939	Tumor	45			Autopsy	Unilateral tumor; bilateral hyperplasia	Testicle filled by cells up to tunica albuginea				Left testicle shows moderate interstitial cell hyperplasia; patient died of carcinoma of cardiac end of stomach
Huffman ¹⁶	1941	Adenoma	6	2 x normal; 5 cm. in diameter	Entire testicle grossly	Surgery	Unilateral	Almost completely encapsulated; irregularly shaped alveolar masses; nuclei round or elliptical; evenly distributed chromatin; large nucleolus	Capsule where tumor does not fuse with albuginea	None; seen 2 years later (hair and gynecomastia still present)	None	Staining property of fat resembles interstitial cells more than adrenal cortical cells; Ponceau stain for androgenic cortical adrenal cells negative; frozen section for anisotropic doubly refractile lipoids negative; deposits of calcium throughout testis; patient had moderate bilateral gynecomastia; Friedman test negative; pubic hair present
Fiablo ¹¹	1941	Tumor	44		2.0 x 2.0 x 1.5 cm.	Surgery	Unilateral	Well defined fibrous capsule; polymorphic interstitial cells; contain one nucleus; cytoplasm contains lipoids and pigment	None	None	None	Benign hydrocele accompanied the testicle
Warren and Olshausen	1942	Tumor	30	4.5 cm. in diameter	2 cm. in diameter	Surgery	Unilateral	Brown-yellow to translucent gray-white in color with punctate gray foci; tumor composed of closely packed interstitial cells	None	None	Present	No seminiferous tubules at site of tumor

one mouse of this strain, 30 days old, by weekly subcutaneous injection of 0.05 mg. of estradiol benzoate.²⁵ Shimkin, Grady and Andervont⁶⁵ implanted 4 to 6 mg. cholesterol pellets, containing 10 to 25 per cent of stilbestrol, subcutaneously into 107 strain C male mice, 6 weeks to 3 months old. Three to 5 months after the implantation of the stilbestrol pellets, the mice developed atrophy of the seminiferous tubules, almost complete lack of spermatogenesis and diffuse hyperplasia of the interstitial cells. Thirteen tumors (nodules of interstitial cells) developed in the 11 months after the implantation of pellets. Mitoses were remarkably infrequent.

HYPERPLASIA OF INTERSTITIAL CELLS IN RELATION TO SPERMATOGENESIS

The infrequency of hyperplasia of interstitial cells described in testes with normal spermatogenesis seems significant, if true. In the group of 33 men from 20 to 70 years who died suddenly, hyperplasia of interstitial cells was found in 2 persons and an unusually large number of cells in 5 others.^{61, 62}

It has often been observed that interstitial cells are increased in conditions associated with diminished spermatogenesis. Many writers have described hyperplasia of interstitial cells with atrophy of seminiferous tubules in chronic debilitating diseases as leprosy, syphilis, tuberculosis, carcinoma, pernicious anemia^{16, 28, 78} and chronic alcoholism.⁷⁸ The increase in interstitial cells described in acute infections, if constant, would be extremely interesting. Cordes¹⁶ studied the testes of 36 patients dying from acute illnesses and found an increase in interstitial cells in 13 of these cases. From a study of 58 persons dying of acute disease, Kyrle³⁸ concluded that the factor determining the occurrence of hyperplasia of interstitial cells was an injury to the parenchyma sufficiently intensive or long-lasting to produce at least a cessation of spermatogenesis. Sehr⁶⁴ distinguished between the hyperplasia of interstitial cells seen in generalized acute conditions and that secondary to carcinoma. He found that with extratesticular carcinoma the interstitial cells were present in large masses up to 1000 cells, which he claimed is much greater than is found in other wasting diseases. Collins¹⁵ found only an insignificant difference in testes of 173 patients with, and a similar number without, carcinoma, and the same was true in a large group of mice.

Abnormality of interstitial cells has occasionally been associated with carcinoma primary in the testis. Pace⁴⁷ and Pace and Cabot⁴⁸ reported 3 examples of carcinoma of the testis in a group of undescended testes. The third case is of unusual interest: two pathologists

thought the testis showed adenocarcinoma, while a third regarded it as extreme tubular hyperplasia. Similarly, the increased interstitial cells were regarded as hyperplastic, premalignant and malignant, respectively.

The interstitial cells may be more numerous in the cryptorchid than in the normal testis,^{24, 28, 53, 67} but this is not always so. The abnormally placed testis naturally varies with the age of the individual, and interstitial cells are said to be rare in the immature ectopic testis.²² Among 24 unilateral undescended testes, all without spermatogenesis, hyperplasia of the interstitial cells was reported in only two: (1) a male of 45 years, the testicle being intra-abdominal (without carcinoma), and (2) one with carcinoma.^{47, 48}

No relationship was demonstrated between disease of the prostate, benign or neoplastic, and the number of interstitial cells in 504 autopsies studied by Teem.⁷⁴ He did find, however, that persons over 69 years of age with hypertrophy of the prostate had relatively fewer interstitial cells than those in this age group with normal prostates.

In our control group of 370 testes, there was no significant relationship between the interstitial cells and the activity of the tubules, the degree of inflammation or the amount of vascular change, which is at variance with the general impression that hyperplasia is accompanied by atrophy of seminiferous tubules. Of the testes of patients with ages known, 21.9 per cent were from persons dead of primary extratesticular malignant neoplasms; 7.7 per cent had primary testicular malignant neoplasms and 1.7 per cent had teratoma of the testis. These did not differ from the others. There were no cases of certain hyperplasia in this control series. Not quite one-third of the testes (111 of 370) showed marked atrophy. Of these 70 cases of known age, only 18.6 per cent had interstitial cells in the upper normal group or possibly slightly above. Of the remaining atrophic testes, about one-half (40 per cent) had interstitial cells in normal numbers and about one-half (41.4 per cent) had few or no interstitial cells. Also, 56 per cent of testes with slight spermatogenesis contained a normal number of interstitial cells. Twenty-seven testes showed marked inflammation, and the interstitial cells were normal in 70 per cent of these. Interstitial cells were below normal in number in our few cases of cryptorchid testes.

Artificially imposed conditions which cause a decrease in spermatogenesis also stimulate proliferation of interstitial cells; *i.e.*, vasectomy, partial castration, testicular graft, changes in environmental temperature, x-ray and radium irradiation,^{15, 38} dietary deficiency, ingestion of drugs and administration of glandular extracts.¹⁵

In animals, an increase in interstitial cells frequently,⁶⁰ but not always,⁷ is found roughly proportional to diminished testicular tubules whether from disease or subnormal development, as in hybrids.⁵⁵ Multiple resections of portions of testes, up to a certain point,³⁶ variation in nutrition,³⁶ x-ray therapy,³⁶ intra-abdominal transplantation,^{8, 38} and acute inflammation induced by intratesticular injection of horse serum 2 weeks after a subcutaneous injection,³² all lessen spermatogenesis and cause increase in interstitial cells. However, the degree of hyperplasia of these cells following x-ray irradiation of testes of dogs seemed out of proportion to the effect on the tubules.³⁸ In rats 21 days of age or older, the interstitial cells were not affected by a diet deficient in vitamin E for a period of from 20 weeks to 9 months. There was atrophy and necrosis of the spermatogenic cells, while the Sertoli cells remained unchanged.²⁹

Hyperplasia of interstitial cells does not necessarily carry any special morphologic variations. Extratesticular cells may be more common in hyperplasia, although this should not be considered an abnormal feature.⁶⁵ Okkels and Sand⁴⁵ described unusually large extratesticular cells, 20 to 50 μ in diameter, and nuclei rather poor in chromatin with one large nucleolus. The cytoplasm stained more deeply, crystals were absent and only very slight pigment was seen. Spangaro⁷⁰ stated that interstitial cells may pass into the hyaline basement membrane, and thence into the atrophic tubules. Common sites for interstitial cells outside the testis are the body and tail of the epididymis,^{4, 42, 56, 76} and along arteries intimately associated with nerves near the corpus Highmori.^{1, 5, 45}

We have collected from the literature 10 cases of hyperplasia of interstitial cells in man. These and the 2 cases we report are presented in Table I. The case reported by Le Chuiton, Prade, Berge and Penhaneac'h³⁹ was classified as adenoma, but we feel it represents hyperplasia, since the interstitial cells were scattered among the cells of a seminoma. The opposite testis was normal. There is only 1 among these 12 cases which might be considered to be hyperplasia in an otherwise normal testis. This is Dürck's¹⁹ third case. Three testes were from hermaphrodites or pseudohermaphrodites and two others were undescended. Atrophy, apparently fairly extensive, was present in three testes. Hyperplasia in 2 cases was associated with tumor. Cleghorn, Hyland, Mills and Linell¹⁴ reported a case of pinealoma with invasion of brain stem and hypothalamus region. The tubules of both testes were completely atrophied and there was hyperplasia of interstitial cells. All of the testes were normal in size or small, except for three which seemed slightly enlarged. Inflammation was

extreme in one testis¹⁰ and prominent in one other (our case no. 2). Extratesticular interstitial cells are reported in only 2 cases; that of Grynfeldt, Truc and Guibert²⁴ from a cryptorchid, and our case no. 1.

These tabulated cases of hyperplasia, while not completely representative of the literature, are important because of the clarity of presentation and nature of the material. The review of our own cases suggests that uncomplicated hyperplasia is extremely rare. A final decision on the significance of association of hyperplasia and other abnormalities must await more exhaustive study of the testis in experimental and pathologic conditions.

LOCAL TUMOR VS. MALIGNANCY OF INTERSTITIAL CELLS

Local tumors collected from the literature are presented in Table II. All of these patients were under 45 years and three of them were under 10 years. These last exhibited precocious sexual development. Stewart, Bell and Roehlke⁷¹ considered the enlargement of the testis in their case as due to premature maturation of the seminiferous tubules rather than to the interstitial cells, although the tumor occupied nearly one-fourth of the testicle. These symptoms could not be ascribed definitely to other endocrine glands. One of the children also had gynecomastia. Gynecomastia was present in one adult. Symptoms regressed after removal of the testis in the children. The tumors were for the most part large and in six instances involved practically the entire testis. In three individuals hyperplasia was present in the opposite testis. Five of the tumors were encapsulated. Chevassu¹² and Kaufmann³¹ described lobulation of the tumor.

The local tumors described in animals are frequently associated with old age and atrophy.^{3, 37, 40, 52, 63} In a series of 59 testes from 48 dogs, for the most part old, Schlotthauer, McDonald and Bollman⁶³ found 51 rather sharply demarcated interstitial cell masses. Only a very small proportion showed normal tubular structure. Aspermia or atrophy was present in the majority. Among 400 dogs examined by Peyron, Blanchard, Poumeau-Delille and Salomon,⁵² 21 had nodular hyperplasia of interstitial cells. Fourteen others had encapsulated tumors; 15 had tumors with more active growth, 2 of which showed change of a malignant type. They reported interstitial cell tumors in horses as well, but these are rare. There was only one hypertrophic nodule and one benign tumor among 275 old animals.

Three cases of malignant interstitial cell tumors in man have been reported: one by Masson and Sencert,⁴¹ the second by de Josselin de Jong,¹⁷ and the third by Masson.⁴⁰ These comprise Table III.

Six cases of neoplasia are controversial. Sacchi⁵⁹ reported his case

TABLE III
Interstitial Cell Carcinomas

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structures	Metastases	Mitoses	Remarks
Masson, and Sencert ¹¹	1923	Interstitial cell cancer	62	8 x 4 x 4 cm.		Surgery	Unilateral (right)	Tubules only beneath the tunica albuginea; tumor: anastomosing cords, 3-4 cells thick, have invaded the surrounding capsule	None	At postmortem: right groin, right femur, right lung, left lung; external iliac, lumbar, and mesenteric lymph nodes; dura mater	Present	Left testicle: benign; normal number of interstitial cells; between degenerated tubules there are voluminous masses; cord shows normal quantity of interstitial cells; first metastases appeared 4 years after appearance of original tumor and 3 years and 10 months after removal of tumor; died 4 years and 4 months after appearance of original tumor
de Josselin de Jong ¹⁷	1926	Interstitial cell tumor: sarcoma arising from Leydig cells; internal masculine pseudohermaphrodite	42	Child's head	Child's head	Surgery	Unilateral	Center: soft, bloody, necrotic; few islands of cells; at edge, cells in bands, rows, or irregular groups		Yes; location not mentioned	None	Left testicle: intra-abdominal; contains "sarcoma." Right testicle: acorn-sized; at external inguinal ring; tubules poorly developed; interstitial cells in groups, largest measures 2-5 mm.; represents a local tumor
Masson ¹⁰ Venning ¹⁵	1942	Interstitial cell tumor	32	5 x 4 x 4 cm.		Surgery	Unilateral	Interstitial cell tumor comprises the corpus Highmori and the atrophic and sclerosed testicular parenchyma; voluminous tumorous nuclei; nodules along venules; early metastases(?)	None	Postmortem: left spermatic cord; liver (7.5 Kg.) (largest nodule, 5 cm. in diameter); lungs, bilateral; dura mater	None	First metastases appeared 10 years after appearance of original tumor and 9 years after removal of tumor; died 10 years after appearance of original tumor; urinary hormone studies (see text)

as teratoma, but Stewart, Bell and Roehlke⁷¹ regarded it as an interstitial cell tumor. Peppere's⁵¹ case of sarcoma of Leydig cell origin was regarded by Jemerin²⁸ as a possible seminoma. Snellman⁶⁰ reported a case as interstitial cell "tumorlike" hyperplasia, but the diagnosis seems doubtful in the presence of many melanin-laden cells in the tumor. Three cases of Stoppato⁷² answer the description for seminoma better than for interstitial cell tumor. Three cases offered insufficient data for classification (Table IV): Villata⁷⁷ reported a case of teratoma, in which there was a considerable number of interstitial cells. Ciceri¹³ mentioned a case of interstitioma. Pitrollfy-Szabó⁵⁴ likewise described a case of interstitial cell tumor without details.

CASE REPORTS OF HYPERPLASIA OF INTERSTITIAL CELLS

Case 1

A. O. was 46 years of age. The left testis was removed because of spontaneous enlargement of 3 weeks' duration. The initial pathologic diagnosis was carcinoma of interstitial cells. X-ray treatment was started soon after operation. A total of 3200 r. units was given, 200 r. units over one area daily to anterior and posterior pelvic areas and 2800 r. units to upper abdomen, anterior and posterior. The following factors were used: 200 kv.; 8 ma.; 50 cm. skin target distance. A urine sample taken for estrin and testosterone 5 weeks after operation was positive for testosterone and negative for estrin. The right testis was thought to be somewhat enlarged and was removed 14 weeks after the first operation. Roentgenograms of the chest taken 1 year later showed a questionable shadow at the base of the left lung. X-ray treatment to the pelvis was repeated approximately 2 years after the first operation. The patient is now well, 5 years after removal of the tumor, without clinical or x-ray evidence of disease.*

The left testis measured 6.5 by 5 by 2.5 cm. The tunics and 3 cm. of the cord were grossly normal. The testis was symmetrical and had a normal shape. Section revealed homogeneous yellowish white tissue with slightly increased consistency and indistinct tubular markings. The external aspect of the epididymis was as usual but the lumen was obscured by fibrous tissue. Microscopic sections were stained with hematoxylin and eosin and with iron hematoxylin-Masson green. Atrophy of the tubules was uniform and almost complete. A few swollen, partly degenerated spermatid cells were still present within the tubules. Besides these, there were cells of different appearance, not definitely identified. Because of some characteristics in common with the interstitial cells, the question of invasion of the tubules by these cells was considered, but could not be definitely answered. The intertubular spaces were filled with rather closely packed polyhedral cells. These cells averaged 12.1 by 9.1 μ in size. The extremes measured 18.5 by 14.9 μ and 8.7 by 8.2 μ . A moderate number of cells had two nuclei and an occasional one had three. These were round, staining lightly with iron hematoxylin, and had one or two nucleoli. No mitoses were seen. Fine, deeply staining granules as well as occasional vacuoles were present in the cytoplasm. Some of the granules stained with light green SF yellowish rather than with iron hematoxylin. Interstitial cells were not found in the tunica albuginea or in the epididymis. A moderate number

* We are indebted to Dr. James E. Waters for the material, and to Dr. Clarence N. McPeak for the x-ray studies.

TABLE IV
Interstitial Cell Growths

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structures	Metastases	Mitoses	Remarks
Villata ¹⁷	1928	Teratoma	22	Larger than 2X normal			Unilateral	Two types of cells: (1) large round with clear cell-body, (2) cells in cords (prevalent)				Teratoma including interstitial cells
Ciceri ¹⁸	1933	Interstitialoma				Surgery						
Pitroffly-Szabó ¹⁴	1937	Interstitial cell tumor				Surgery						

TABLE V
Local Tumor with Interstitial Cell Hyperplasia

Author	Year	Diagnosis	Age in years	Size of testicle	Size of tumor	Surgery or autopsy	Unilateral or bilateral	Character of tumor	Involvement of adjacent structure	Metastases	Mitoses	Remarks
Warren and Olshausen	1942	Local tumor plus interstitial cell hyperplasia	44	Small (bilateral)	Same as testis	Autopsy	Bilateral	Increased number of interstitial cells between tubules	Tunica albuginea	None	None	Tumor arising from hyperplasia; in some areas seminiferous tubules are present, while in others, they have been destroyed

of lymphocytes were interspersed among the interstitial cells and there were also occasional collagen strands.

The right testis measured 4 by 3 by 2.5 cm. The contour of the testis, epididymis and 2.2 cm. of cord was not unusual. The tunica was slightly thickened by fibrous tissue and there was evidence of old fibrin toward the lower pole. Adhesions obliterated the sac of the tunica vaginalis in part and there was slight extension of fibrous tissue into the testis. This fibrosis was especially marked adjacent to the epididymis. The bulk of the testis approximated normal, but the tubules would not string out appreciably. The epididymis was almost entirely fibrosed. The microscopic sections showed some atrophy, but this was less marked than in the left testis. There was no spermatogenesis and the Sertoli cells were edematous and vacuolated, the nucleus having been displaced to the base of the cell. Interstitial cells were present in moderate numbers between the tubules. Their appearance did not vary greatly from the normal. They were polyhedral. The cytoplasm was finely granular with occasional small vacuoles. The nucleus, occupying one-third to one-half of the cell, was also finely granular. Double or triple nuclei were not unusual and nucleoli varied from one to three. A few interstitial cells were oval or elongated, suggesting primitive mesenchymal cells. Interstitial cells were most numerous in the region of the tubuli recti and sheets of them lay between the coils of tubules in the epididymis. There were a few mitotic figures in these latter sites. An inflammatory reaction with fibroblasts and infiltrating cells, chiefly lymphocytes with a scattering of polymorphonuclears, was fairly conspicuous in the stroma throughout the testis. This reaction was present in about the same degree in the epididymis.

Case 2

A. S. was 51 years of age. A radical excision of the external genitalia was done for carcinoma of the penis following observation over 12 years for multiple sinuses and strictures of the urethra. The left testis measured 3.5 by 2 by 1.8 cm. and the right, 1.7 cm. in greatest diameter. The latter contained somewhat more fibrosis than the left testicle. The portion of the right testis near the globus minor was more yellow and less firm than the rest of the testis. Microscopically, both testes were atrophic, especially the right. In both, there were large aggregates of from 25 to 100 interstitial cells. The average measurements of the cells were 14.7 by 9.1 μ . The extremes of size were 21.9 by 11.2 μ and 9.1 by 7.9 μ . Since the tubules had not been replaced by interstitial cells, the increased number of those cells in both testicles has been regarded as interstitial cell hyperplasia.*

CASE REPORTS OF TUMOR OF INTERSTITIAL CELLS

Case 3

E. B., 44 years of age, had generalized lymphoblastoma of 5 years' duration. Small doses of x-ray had been given to the lymph nodes in various locations during that time, but the testes were never exposed. At autopsy, both testes were atrophic. There was marked atrophy of seminiferous cells and hyaline thickening of the basement membrane. Large islands of interstitial cells separated the tubules and in part appeared to replace them. The cells extended slightly along the outer sheath of blood vessels into the tunica albuginea. No mitotic figures were present. In view of the character of the cells and the absence of metastases, this growth cannot be considered malignant, although some aggregations of interstitial cells had overgrown focally the seminiferous tubules and destroyed them. The fairest interpretation seemed to be local tumor associated with hyperplasia.

* We are indebted to Dr. Loring H. Raymond for the material of this case.

Case 4

J. R. was 30 years of age. This patient presented himself to his physician complaining of digestive disturbances. On physical examination, a tumor was found in the right testis. The initial pathologic diagnosis was interstitial cell carcinoma. This was said to have been present for 6 weeks. A right orchidectomy was performed. Following the operation, chest films and Aschheim-Zondek test were negative. The patient is now symptomless, 2 years after orchidectomy.

The testis measured 4.5 cm. in greatest diameter. In the upper pole a discrete tumor 2 cm. in diameter differed in color and consistency from the rest of the testis which approximated normal. This tumor was composed of densely packed polyhedral cells averaging 14.9 by 11.6 μ . The extremes measured 23.1 by 18.8 μ and 11.0 by 8.4 μ . The cells differed from the normal interstitial cells in being larger. Their cytoplasm contained densely packed fine acidophilic granules and fine vacuoles. Occasional mitotic figures were seen. There were no tubules within the tumor and those at the periphery were compressed. The remainder of the testis was not unusual.* We consider this a local tumor of interstitial cells.

DISCUSSION OF CASES

A study of the tabulated human cases † of hyperplasia, tumor and malignant neoplasia reveals the following:

Age. Fifty-seven per cent of the cases of hyperplasia occurred at or above 45 years of age, while all of the local tumors occurred at or under 45 years of age. Two of the three cases of malignant neoplasms occurred under 45 years of age.

Size of the Testicle. The data for hyperplasia are too indefinite for comparative purposes; however, the testis is usually small. In contrast, fifty-seven per cent of the local tumors were one to two times the size of a normal testicle, while 19 per cent were normal in size or less.

Surgical or Postmortem Material. Fifty-eight per cent of the examples of hyperplasia were obtained at autopsy, while 85 per cent of the tumors were obtained by surgical procedures. The malignant tumors were removed surgically.

Involvement of Adjacent Structures. Of the 28 cases, 3 cases of hyperplasia showed involvement of the adjacent structures; namely, the atrophic tubules, tunica albuginea and epididymis, respectively.

Metastases. Metastases were found in three cases, one of which showed diffuse involvement of the testicle with no tubules remaining. One tumor was surrounded by a capsule, and in the third case, tumor was found along small blood vessels and was suspected of being possibly early metastasis. None of the testicles with solid tumors or so-

* We are indebted to Dr. Earl E. Ewert of the Lahey Clinic for the clinical data.

† One case, which showed histologic characteristics of both hyperplasia and local tumor, was not included in the series for statistics. This case is tabulated separately (Table V).

called hyperplasia presented any metastases. In only one of these were tubules absent, and this patient lived for at least 22 months post-operatively.

Mitoses. Three cases of hyperplasia, one local tumor, and only one of the three malignant tumors showed mitoses.

Ectopic Testicles. Four of the testicles with hyperplasia and one of those with malignant neoplasms were ectopic.

Malignant Neoplasms. One of the malignant tumors occurred in the age group (above 45 years) in which hyperplasia is most common, while the other two occurred in the age group in which local tumor is most common. De Josselin de Jong's case¹⁷ was ectopic. Mitoses, found in one of the malignant neoplasms, were found also with hyperplasia and local tumor. In Masson and Sencert's case⁴¹ as well as in that of Masson⁴⁰ the malignant tumor may have arisen from a local tumor, as only a few interstitial cells were seen among the few tubules in the first, and the tumor was compressing the tubules in the second case.

One of Dürck's¹⁹ examples of hyperplasia showed irregular cells and atypical mitoses, and appeared similar to sarcoma.

It is thus seen that, to date, no definite criteria for malignancy can be named, except the presence of metastases. However, it appears that a patient whose testicle contains an increased number of interstitial cells with or without mitoses must be carefully watched for the occurrence of metastases. It is known that a latent period of 9 years may occur between the removal of the original tumor and the clinical appearance of metastases.⁴⁰

The great variation in the descriptions of interstitial cell tumors in man as given by various authors is rather difficult to understand. Among the varied pictures a resemblance to cords of seminiferous tubules and to adrenal cortical malignancies has been described.

Hormones. Hormonal determinations in patients with interstitial cell growths have been few. In the case reported by Masson⁴⁰ and Venning,⁷⁵ the 24-hour urinary output of gonadotropic and estrogenic substances was 110 and 113 mouse units per day, respectively (slightly elevated above normal). The 24-hour urinary 17-ketosteroid level was elevated fifty times above the normal mean. Three determinations made in the presence of metastases 1 to 2 months before death were evaluated at 980, 1035 and 1040 mg. per day. These are the highest 24-hour urinary 17-ketosteroid determinations ever recorded, including those for adrenal tumors.⁷⁵ An hydrolysis of urine revealed androsterone sulfate and androstenone. The serum 17-ketosteroid level was 16 mg. per cent.

SUMMARY

Too little is known of the normal variation of interstitial cells to differentiate clearly pathologic states of these cells. On the negative side, there are certain helpful facts:

1. Large collections of interstitial cells are not necessarily pathologic.
2. Multinucleated cells, and cells in the tunica albuginea and epididymis do not necessarily indicate invasive growth.
3. Cyclic changes in the number of interstitial cells from fetal life to senility tend to correspond inversely with changes in spermatogenesis.

Hyperplasia of interstitial cells as found in chronic diseases, injury, castration, cryptorchidism, changes in environment, ingestion of drugs in man, and exposure to x-ray and radium, transplantation of testicles, administration of glandular extracts (prolan B and estrogens) and serum in animals may be due entirely or largely to the diminished spermatogenesis. Of 29 reported human cases of increase in number of testicular interstitial cells, including 4 cases of our own, 12 were classified as hyperplasia, 13 as local tumor, 1 as local tumor accompanied by hyperplasia and 3 as malignant.

The criteria for this classification have been presented.

Hyperplasia occurs principally at or above 45 years of age in atrophic testicles and is discovered chiefly at autopsy. Local tumors occur predominantly at or below 45 years of age in testicles larger than normal, and are generally discovered during life and removed surgically.

Two of the three malignant tumors occurred below 45 years of age.

No definite criteria for malignancy can be named, except for the presence of metastases. However, if the testicle of a patient contains an increased number of interstitial cells, especially if the seminiferous tubules have been partially or completely destroyed, he should be carefully followed for more than 10 years for the appearance of metastases, if not present before that time.

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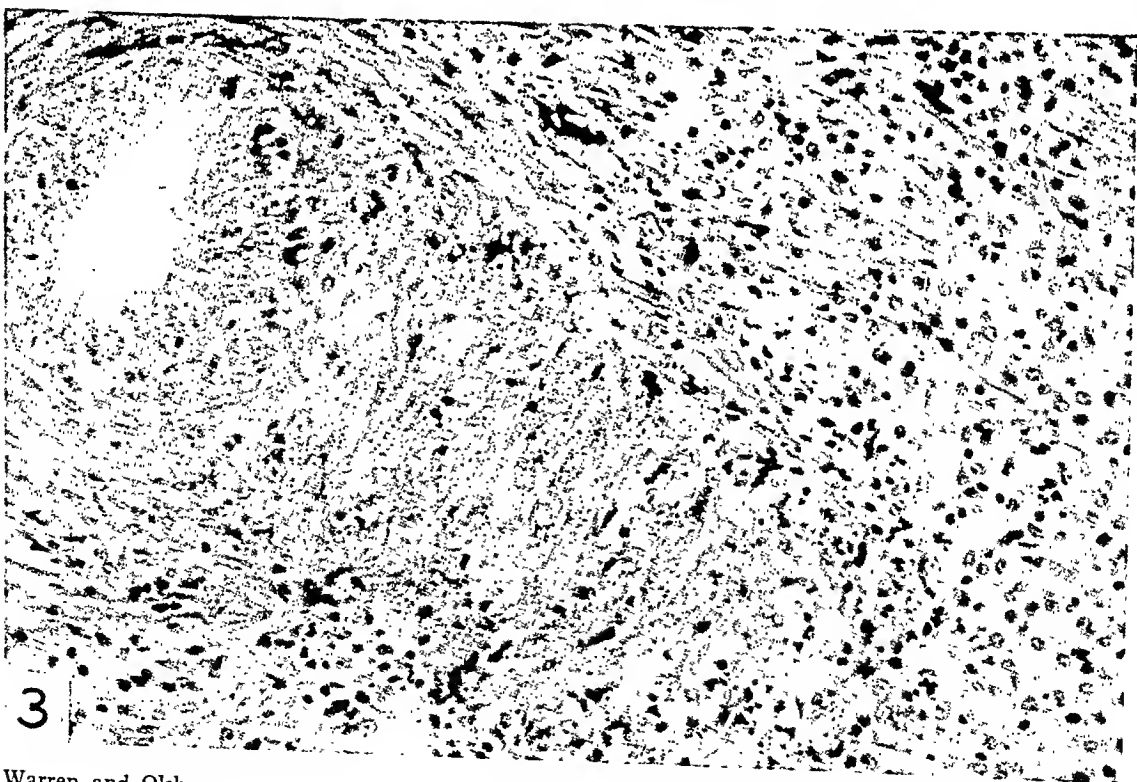
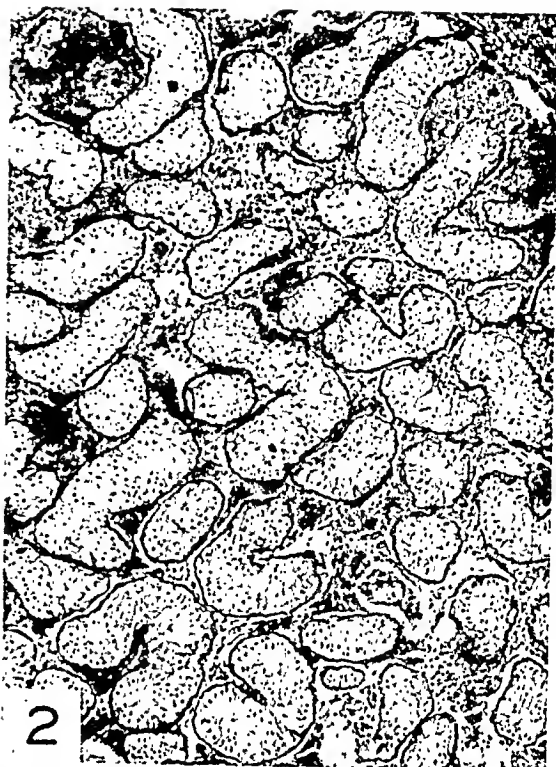
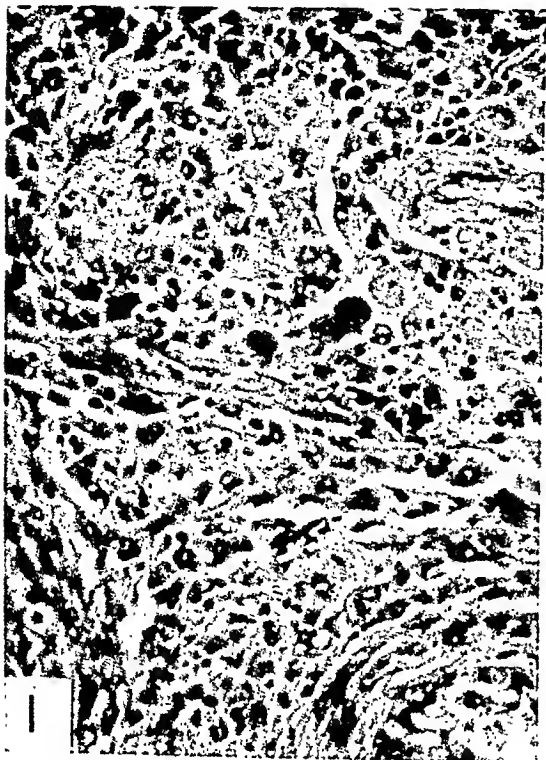
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DESCRIPTION OF PLATES

PLATE 37

- FIG. 1. Case 1. Interstitial cells in first testicle removed. A portion of an atrophic tubule is shown in the lower right corner. $\times 235$.
- FIG. 2. Case 1. Atrophic seminiferous tubules and increased interstitial cells in second testicle removed. $\times 45$.
- FIG. 3. Case 1. Epididymis of second testicle removed, showing large number of interstitial cells. $\times 235$.



Warren and Olshausen

Interstitial Cell Growths of the Testicle

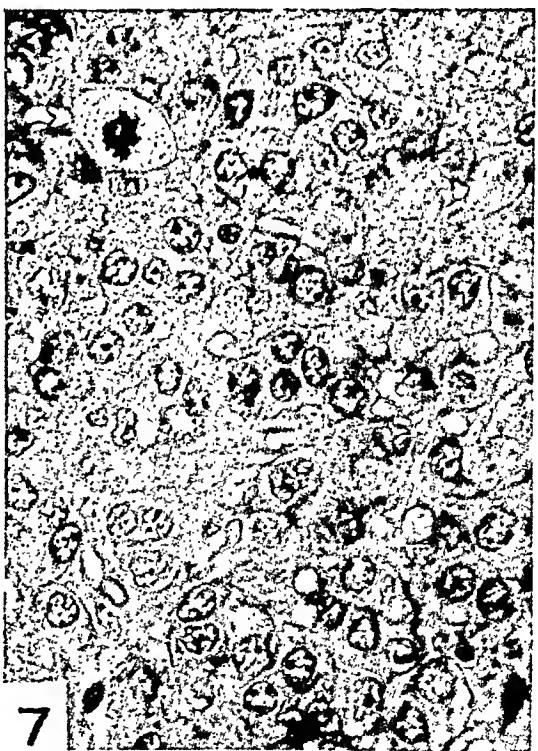
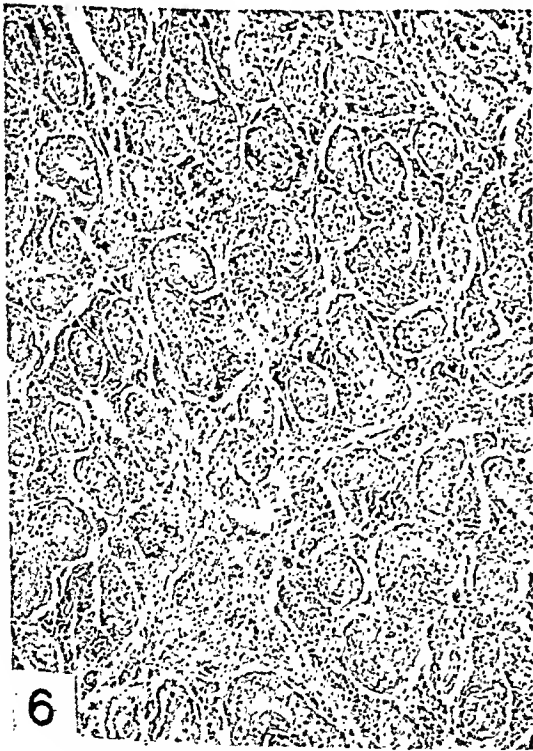
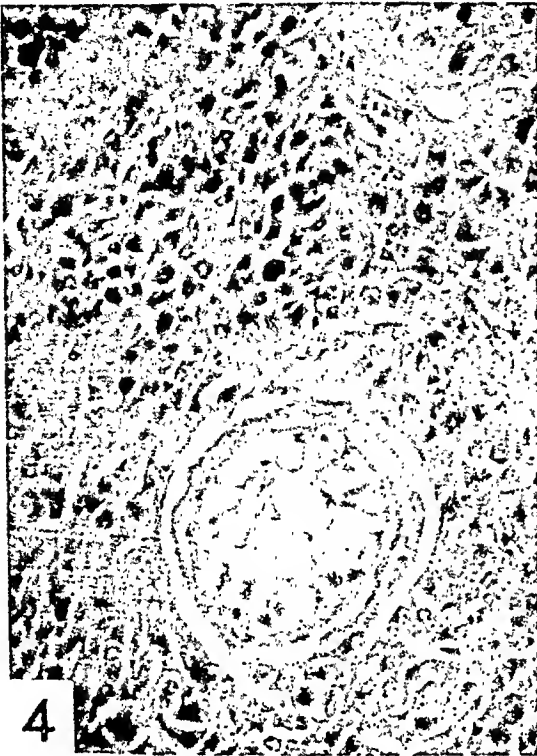
PLATE 38

FIG. 4. Case 3. Interstitial cells and atrophic seminiferous tubule. $\times 235$.

FIG. 5. Case 3. Interstitial cells and atrophic seminiferous tubules. There is a marked diminution in the number of tubules in the lower half. $\times 45$.

FIG. 6. Case 2. Increased interstitial cells between atrophic seminiferous tubules of left testicle. $\times 45$.

FIG. 7. Case 4. Interstitial cells in tumor with mitotic figure. $\times 470$.



Warren and Olshausen

Interstitial Cell Growths of the Testicle

FOCAL GLOMERULITIS IN ELDERLY PATIENTS *

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In a previous report¹ it was established experimentally that severe reduction of functioning renal parenchyma may be associated with proliferative glomerulitis and the hypothesis was suggested that the production of these glomerular lesions was caused by excessive work demand placed upon the glomeruli.

The present study was undertaken to determine the frequency of this type of glomerulitis in persons 60 years of age or older since reduction in the number of glomeruli is a natural accompaniment of senescence.²

METHOD

One hundred and seventy-four cases were selected from the autopsy file of The Western Pennsylvania Hospital. All patients were 60 years of age or older. None had diffuse glomerulonephritis or clinical evidence of pyelonephritis. In many instances only one slide was available for study. Only those cases were included in which the sections were technically satisfactory. Where paraffin blocks were available in cases showing severe involvement, additional sections were prepared and stained with azocarmine.

One hundred or more glomeruli were studied in each case and an estimation made of the percentage of glomeruli with inflammatory changes. Altered glomeruli in regions of scarring or of cellular infiltrations were not considered pertinent. Glomeruli which showed pericapsular fibrosis were likewise excluded. The cases were arbitrarily graded according to the incidence of glomerulitis: from 0 to 9 per cent, negative; from 10 to 24 per cent, slight; from 25 to 49 per cent, moderate; and 50 per cent or more, severe.

Autopsy records and clinical charts of the cases with severe involvement were consulted in an attempt to correlate the renal changes with cardiac weights and with the presence or absence of hypertension or uremia.

For a control series, 46 patients between the ages of 17 and 38 years were selected similarly from the autopsy file and the kidney sections studied with the same criteria in mind.

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RESULTS

Description of Glomerular Alterations

In well defined cases even a cursory examination of the sections indicated an increased cellularity of the glomerular tufts (Fig. 1). This abnormality constituted the most frequently encountered alteration. Admittedly, the decision whether the cellularity in any instance is increased or not is arbitrary. However, in this study only those glomeruli were considered of increased cellularity in which the increase in the number of cells did not appear questionable. In reaching this decision an absolute increase in cellularity was differentiated from the relative increase in cellularity caused by collapse of capillaries.

As indicated by the azocarmine stain, the increased cellularity was in some instances caused almost exclusively by an increase in epithelial cells. In other glomeruli principally the endothelial cells were increased in number. However, more often cells of both types were more or less equally increased in number. The distribution of nuclei throughout the glomerulus was not always uniform. "Bunching" or crowding of nuclei as noted in the rat ¹ was also seen in a few of the glomeruli. No pyknosis was observed. The nuclei appeared normal. Focal necrosis was not found.

A second notable alteration, evident also on cursory examination, was a more solid and rounded, "simplified" appearance of many of the glomeruli. These glomeruli differed appreciably from sclerotic glomeruli which, although also solid and simplified, were contracted, relatively acellular and possessed obliterated capillaries. The type of glomerulus under discussion was of average size or frequently greatly enlarged, the cellularity increased, and the capillaries clearly discernible, although often empty of blood. The solid appearance of the glomerular tufts was caused by a pronounced increase in cytoplasmic constituents. Both epithelial and endothelial cells were greatly enlarged. The cytoplasmic bodies of endothelial cells frequently occluded capillary lumina and the cytoplasm of epithelial cells was at times even more abundant. The epithelial cells seemed to cement adjoining glomerular loops together and to fill the spaces between them. Thus, the complex outline of the tufts was obliterated and "simplification" produced.

The endothelial and epithelial cytoplasm of the glomerular tufts was frequently of hyaline character and, in azocarmine-stained sections, was a delicate purple or, at times, a very intense, homogeneous dark blue which was continuous with the dark blue-stained substance of

the basement membrane. Only rarely were distinct cytoplasmic granulations seen which corresponded to the hyaline cytoplasmic granulations described by McGregor.³ In azocarmine-stained preparations the blue-staining cytoplasm of epithelial and endothelial cells often contributed to an irregular thickening of the capillary basement membrane. However, many of the affected glomeruli showed little or no thickening of the capillary basement membrane. Intracapillary fibrillae were not observed.

Proliferation of capsular epithelium was another prominent feature which was found either with or without proliferative changes in the tufts. In a few glomeruli the nuclei of the capsular epithelium presented the appearance of a chain of closely approximated beads (Fig. 2). In addition to hyperplasia there was at times also hypertrophy of the capsular epithelium which assumed cuboidal or even tall columnar forms. These capsular epithelial changes were not associated with pericapsular fibrosis.

An occasional section exhibited one or two glomeruli in which focal adhesions were present between tuft and capsule (Fig. 3). These adhesions were epithelial in character and were not associated with fibrous tissue proliferation in either the tuft or the capsule. All changes were generally greatly exaggerated in and near foci of pyelonephritis.

Incidence of Glomerular Alterations in the Elderly Group and Clinicopathologic Correlation

The proliferative glomerular changes were usually mild in degree. About one-half of the cases showed no significant involvement. In 20 per cent of the 174 cases the glomerular involvement was classified as slight; in 13 per cent, as moderate; while in 30 cases, or 17 per cent, the changes were more extensive and severe. The weight of the heart was indicated in 27 of the latter (permission to examine thoracic organs had been withheld in the other 3). In 16 of the 27 cases there was cardiac hypertrophy (375 to 540 gm.). Many of these patients were hypertensive and some were uremic. The cardiac weights in the other 11 cases ranged between 225 and 350 gm. and although the blood pressure was not charted in all, when recorded it was within normal limits. Similarly, chemical determinations upon the urine and blood showed no significant elevation in these cases. Nephrosclerotic changes were prevalent in most of the cases but were least in evidence in the 11 which showed severe proliferative glomerular involvement and no cardiac hypertrophy.

Incidence of Glomerular Alterations in the Control Group

Only 11 per cent of the controls (5 of 46 cases) showed glomerular changes comparable to those in the preceding series. The most severe changes were found in a white male, 36 years old, who died of hepatic cirrhosis with ascites. The glomerular tufts were smaller than average and showed increased cellularity with pronounced hyalinization of the proliferated cells. They were somewhat similar to the changes described by Horn and Smetana⁴ in a case of hepatic cirrhosis.

There were 3 cases with moderate glomerular involvement:

(a) A white male, 28 years old, who died of leptomeningitis and bronchopneumonia. Although the blood pressure was not recorded, the earlier existence of hypertension seems probable in view of a considerable cardiac hypertrophy (425 gm.) in the absence of valvular, or other cardiac defects. (b) A white male, 29 years old, who died of spongioblastoma multiforme. There was subclinical pyelonephritis. The heart weighed 300 gm. (c) A white male, 35 years old, who died of gastric carcinoma with metastases. The blood pressure was 180/130 and the cardiac weight was 350 gm.

The fifth case showed relatively milder glomerular involvement. This was a white women, 32 years old, who died of bronchopneumonia. She had mitral stenosis and the heart weighed 375 gm.

Proliferation of capsular epithelium was surprisingly common (22 per cent) in the control group. However, it was not nearly so pronounced or so widespread as in the older age group.

DISCUSSION

Inflammatory glomerular lesions identical with those found in diffuse glomerulonephritis have been described by McGregor³ in essential hypertension and by Kimmelstiel and Wilson⁵ in hypertension and pyelonephritis. The latter described many of the histologic features found in the present study under the term "alterative" glomerulitis. There are, however, a number of features which are different. According to Kimmelstiel and Wilson⁶ the lesion is primarily a degenerative one and is associated with focal necrosis, and adhesions of tuft to capsule are of fibrinous character. Proliferative changes are considered secondary and may even be associated with exudative processes. McGregor described capillary loops occluded by inflammatory monocytes and hyaline fibers in addition to the other features, but made no mention of necrotic foci. She considered hyaline degeneration of epithelium secondary and a sequence to proliferation.

There appears to be little doubt that the glomerular changes described in this paper are of the same type as those described in a previous experimental study¹ and by others.⁷⁻⁹ Existing differences

in interpretation, emphasis and minor detail are inevitable and understandable. These glomerular lesions have been linked with the hypertensive and the uremic states.^{3, 5, 10} Kimmelstiel and Wilson⁵ found such lesions in 13 of 56 cases of pyelonephritis. Only 1 of the 13 was not hypertensive. All had renal insufficiency.

Kimmelstiel and Wilson¹⁰ stated that "renal impairment cannot be excluded as an etiologic factor" and also that "in view of the inconclusive character of the evidence, however, we still entertain the possibility of an extrarenal toxin which may produce vascular spasm with coincident renal failure and alterative glomerulitis." The present study indicates that the glomerulitis under discussion is not necessarily associated with either the uremic or the hypertensive state because it has been found with considerable frequency in 11 persons who had neither hypertension nor clinical evidence of renal impairment. The results here presented give added support to the hypothesis that pronounced reduction in functioning renal parenchyma and the resulting excessive work demand placed on the remaining glomeruli is a probable etiologic factor of these glomerular lesions.

An assumption of reduction of renal parenchyma as an etiologic factor of the glomerular lesions in the control group (11 per cent) does not appear valid. The youth of these patients and the absence of scarring or serious active inflammation in the kidneys precludes such assumption. This suggests that the type of glomerular damage under discussion may also be produced by a number of other conditions, among which may be hypertension,^{3, 5, 10} chronic pyelonephritis,⁵ and possibly others.

One of the puzzling features in differentiating histologically between advanced chronic glomerulonephritis and certain cases of advanced nephrosclerosis (benign hypertension) has been the presence of glomerulitis in the latter which, as McGregor³ has pointed out, may be identical with that seen in the former. The question has arisen whether there is a superimposed glomerulonephritis in such cases of nephrosclerosis. The recognition of the glomerulitis under discussion as an accompaniment of hypertension, uremia, or senescence seems to offer an attractive explanation to an otherwise confusing picture.

SUMMARY

1. Inflammatory glomerular changes were found in about one-half of 174 patients of 60 years or older who did not have glomerulonephritis or clinical evidence of pyelonephritis. Only 11 per cent of the patients between the ages of 17 and 38 years exhibited such lesions.
2. The glomerulitis was of proliferative character, involving the epithelium of the tuft and capsule and also the capillary endothelium.

Occasional adhesions between tuft and capsule, and hyaline degeneration of epithelium and endothelium were other important findings.

3. The frequency of the glomerulitis was slight in 20 per cent of the cases, moderate in 13 per cent and severe in 17 per cent (30 cases). In 11 of the 30 patients with severe involvement there was no hypertension or clinical evidence of uremia.

4. These findings are interpreted as supporting the hypothesis that the genesis of such lesions in some of these patients is related to a pronounced reduction in functioning renal parenchyma and that it is probably dependent upon excessive work demand placed on the remaining glomeruli.

5. Other conditions may also cause the proliferative glomerulitis under discussion. These may include hypertension and chronic pyelonephritis.

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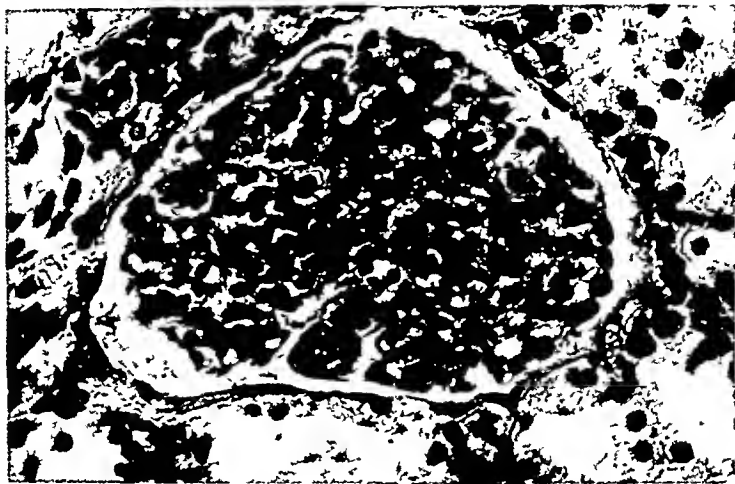
DESCRIPTION OF PLATE

PLATE 39

FIG. 1. Glomerulus showing marked increased cellularity and simplified appearance of the glomerular tuft with a normal capsular epithelium. Hematoxylin and eosin stain. $\times 290$.

FIG. 2. Glomerulus showing proliferation of the capsular epithelium with a beadlike arrangement of the nuclei. The glomerular tuft is normal in appearance. Hematoxylin and eosin stain. $\times 290$.

FIG. 3. Glomerulus showing proliferation and swelling of the glomerular epithelium in the upper left portion of the tuft and an adhesion between the tuft and capsule in the upper right portion of the tuft. Hematoxylin and eosin stain. $\times 290$.



1



2

Gross and Morningstar



3

Focal Glomerulitis in Elderly Patients

DEVELOPMENT OF MYOCARDIAL NECROSIS AND ABSENCE OF NERVE DEGENERATION IN THIAMINE DEFICIENCY IN PIGS *

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Cardiac failure is well recognized as a part of the syndrome of beriberi in man. The nature of the physiological disturbance leading to such failure has not been well understood and the character of the anatomical changes in the heart itself has been a matter of dispute. Deficiency of the thermolabile portion of the vitamin B complex has long been recognized to be the chief cause of beriberi but, until recently, animal experiments in which a deficiency of this part of the B complex was produced have thrown little light on the cardiac aspects of the disorder.

In several recent reports, however, pathological changes have been described in the myocardium of pigeons,¹ dogs² and pigs³ in which deficiency was produced by feeding autoclaved, or sodium sulfite-sulfur dioxide treated, diets. The changes noted have been attributed to lack of thiamine, both because this has been regarded as the chief or only vitamin of the B complex injured by such treatment of the diet and because improvement followed its administration. Thiamine therapy has also been described as dramatically alleviating the symptoms and signs of cardiac embarrassment in certain cases of cardiac failure in man.^{4, 5}

As we have pointed out elsewhere,⁶ experiments which depend for the elimination of thiamine on procedures designed to destroy it are open to criticism since, in the process of destruction, other vitamins may be affected as well. Our own studies of thiamine deficiency were not commenced until crystalline vitamins of the B group became available in such quantities and variety that it was possible to secure good growth and development by feeding a vitamin B-deficient diet supplemented only by crystalline vitamins.⁷ Although it must be recognized that our basal diet, even when supplemented in this way, may still be incomplete, it is important to note that thiamine deficiency was produced by omitting or reducing the amount of this vitamin in the dietary supplements rather than by resorting to a destructive pro-

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† Upjohn Fellow in Medicine.

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cedure which could possibly also destroy other factors than thiamine.

A detailed description of the effects of thiamine deficiency on the state of health, the urinary thiamine excretion and the blood pyruvic acid in young pigs has been given elsewhere.⁶ We wish to present here the details of the pathological changes found in the hearts of the experimental animals and to point out again our failure to detect any pathological changes in the nervous system.

MATERIAL AND METHODS

The present study deals with observations on two groups of pigs given crystalline vitamins and one group fed desiccated whole liver instead of these vitamins. The first group (1, "thiamine omitted"), were given riboflavin, 0.12 mg.; nicotinic acid, 1.20 mg.; pyridoxine hydrochloride, 0.20 mg.; choline chloride, 10.0 mg., and calcium pantothenate, 0.50 mg., per Kg. of body weight per day. The second group (2, "low thiamine"), were given 10 μ g. thiamine hydrochloride per Kg. of body weight per day in addition to the above crystalline vitamins. The third group received none of these vitamins but were fed 1.5 gm. of desiccated whole liver per Kg. of body weight per day. This quantity contains about 40 μ g., or less, of thiamine but is rich in all the other components of the B group.

Full details of the experimental procedure are found elsewhere.⁶⁻⁹

When the animals died or were killed, complete autopsies were performed. Blocks of all the tissues were fixed in formaldehyde and in one animal from each group all the tissues were imbedded in paraffin and stained with hematoxylin and eosin. In addition, the heart and lungs were carefully examined in all the animals, as were also the brachial, sciatic and vagus nerves, the sensory and motor nerve roots, the dorsal root ganglia and the spinal cord. The brains of three representative animals were also studied.

For comparison with these 9 animals we have examined sections of the myocardium of 45 pigs, some of which had received all the crystalline vitamins of the "B" group⁷ while others had been deficient in one or more components of this complex.^{7, 9} A number of these animals failed to grow normally and two were pigs whose food had been purposely restricted to study the effect of chronic inanition on the tissues.⁸

OBSERVATIONS

Group 1

Pig no. 6-53. This animal died after an experimental period of 156 days. During this time three episodes of vomiting, anorexia and loss of weight had occurred; accompanying these symptoms there was an elevation of the pyruvic acid level in the blood. The first two episodes

were relieved by the administration of thiamine and the blood pyruvic acid decreased to normal values. During the last 3 weeks of life, which was maintained by giving small and inadequate amounts of thiamine, the pyruvic acid level of the blood was constantly elevated. Vomiting and anorexia developed in the last week of life. On the day of death the animal was observed to become weak and short of breath. It walked reluctantly and unsteadily. The heart sounds were of good quality. Cyanosis developed suddenly and was soon followed by loss of consciousness and death.

The carcass weighed 63.8 Kg.; the heart, 280 gm. or 0.44 per cent of the body weight. The heart was dilated and the musculature was pale and flabby. In the myocardium of the left ventricle there were numerous fine yellow streaks several millimeters in greatest diameter. The valves were normal. The lungs were hemorrhagic and edematous. The other organs were not remarkable.

Microscopically, there were widespread lesions in the auricles and ventricles. These consisted of areas of necrosis of muscle fibers. Such foci were infiltrated by polymorphonuclear neutrophils, a few eosinophils and monocytes. In the older lesions there were connective tissue cells as well. The initial change seemed to be a loss of striation accompanied by vacuolization and hyalinization of the fiber; leukocytes then appeared. The lesions varied from small foci (Fig. 3) involving only a few fibers to large areas covering several low-power microscopical fields (Fig. 2). The latter were undoubtedly the areas seen grossly. In older lesions no intact muscle fibers could be found; here the predominant cells were mononuclear, consisting of macrophages and connective tissue cells. Scattered among these in much smaller numbers were neutrophils and eosinophils. No large necrotic areas were encountered in the auricles, where the lesions consisted of minute focal necroses. The latter were widespread. In some of the intact, normal appearing fibers vacuoles could be seen.

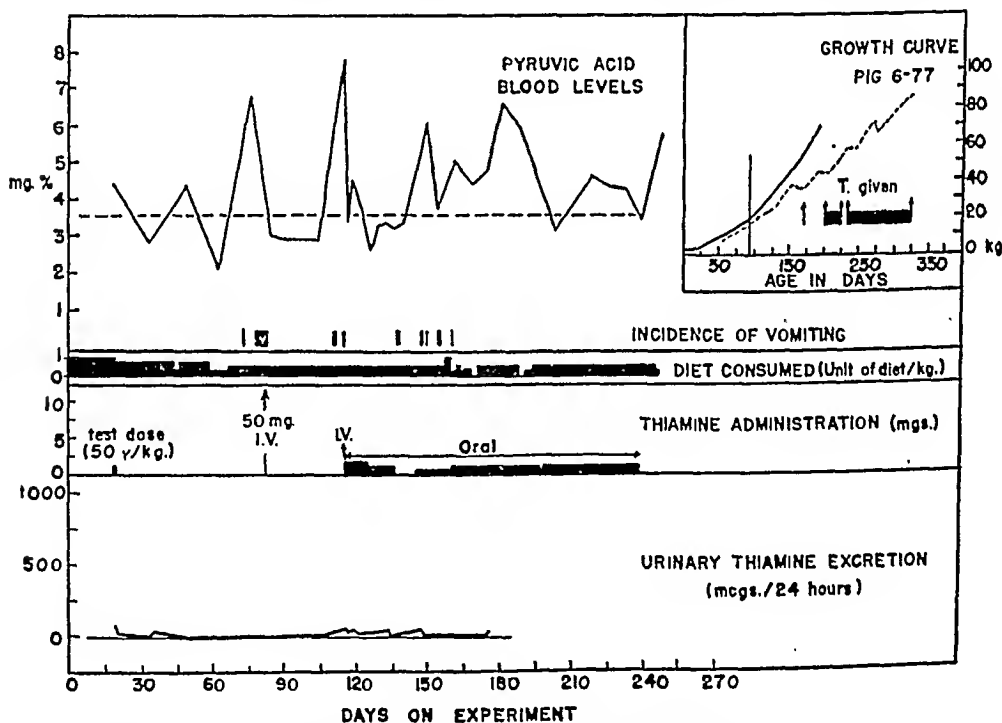
In the pulmonary alveoli, red blood cells and fluid could be found. The liver showed no chronic passive congestion. Histological examination of the nervous tissues revealed no lesions.

Pig no. 6-67. This animal lived 83 days on the diet free of thiamine. No ill effects were observed until the deficiency had lasted 74 days, when the animal began to vomit. On the 75th experimental day the content of pyruvic acid in the blood was found to be very high (6.8 mg.), and 8 days later the animal was found dead. It is of interest to point out that the pyruvic acid level of the blood had been somewhat elevated (4.9 and 4.5 mg.) or at the upper limits of normal (3.5 mg.) for the last 47 days and had been very high for about 1 week; thiamine was administered on only one occasion, 64 days before death.

The heart was not weighed. Microscopically, only a section of the left ventricle was available for study. Here were areas in which there was diffuse leukocytic infiltration between the muscle bundles; some of the latter were necrotic. In contrast to pig no. 6-53, there were no large localized lesions.

The lungs were edematous. The other tissues were autolyzed. Study of the nervous tissues revealed no degeneration.

Pig no. 6-77. This animal died after an experimental period of 246 days. During the midportion of this period there were numerous episodes of vomiting and anorexia, accompanied by elevations of pyruvic acid in the blood (Text-Fig. 1). Thiamine was administered from time to time to decrease the severity of the deficiency. The pyruvic acid content of the blood had remained elevated most of the time for the last 100 days. During the final week no thiamine was given and the pyruvic acid in the blood increased to 5.7 mg. At this time the animal appeared listless and was reluctant to move about.



Text-Figure 1. Growth, incidence of vomiting and anorexia, content of pyruvic acid in the blood and urinary thiamine excretion in a pig (no. 6-77) fed the basal diet supplemented only with riboflavin, nicotinic acid, pyridoxine, choline and pantothenic acid. Thiamine was given only as a "test dose," as a temporarily curative dose of 50 mg., and later only in subminimal doses (20 to 30 μ g. per Kg. of body weight daily). The latter were furnished in order to produce a chronic deficiency of thiamine.

The interrupted line in the section representing pyruvic acid indicates the upper limit of pyruvic acid in animals not deficient in thiamine.⁶

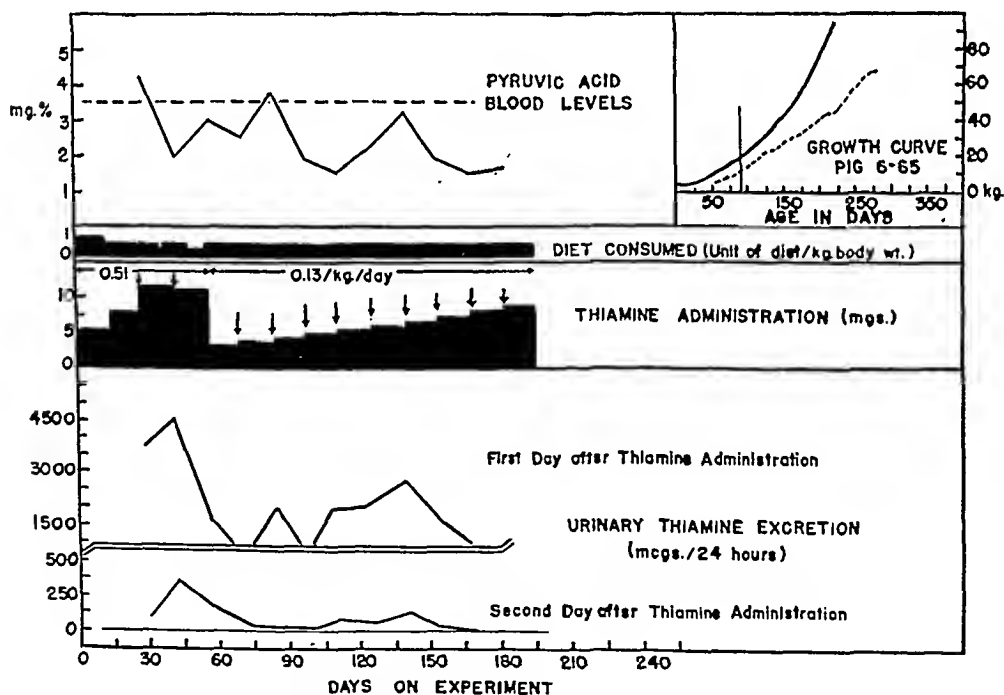
The continuous line in the growth curve represents the growth of pigs given a mixed diet at the Beltsville Research Center and fed and handled according to record of performance procedure. The interrupted line indicates the growth of the animal studied.

As it was exercised, marked dyspnea and pronounced cyanosis developed. With deepening of cyanosis the animal collapsed and, after renewed activity, passed into coma following a series of convulsive movements. Death occurred about 1 hour later.

The carcass weighed 84 Kg. The heart, which was dilated, weighed 500 gm. (0.60 per cent of the body weight).

Microscopically there were numerous lesions scattered throughout both auricles and ventricles. They were more extensive in the right auricle than in the left. In the ventricles there were focal areas of necrosis, some of which covered a quarter of a microscopical field. There were fresh, smaller foci as well. In addition to the areas of necrosis associated with leukocytic infiltration, there were many places where the muscle fibers had disappeared and connective tissue had taken their place. Such foci were interpreted as being areas which had formerly been necrotic and were now healed.

The lungs showed edema. The hepatic sinuses were engorged with blood but there was no atrophy of the central liver cells. Examination of the nervous tissue, including the brain, revealed no abnormalities.



Text-Figure 2. Growth, diet consumption, content of pyruvic acid in the blood and urinary thiamine excretion in an animal given thiamine in adequate amounts in addition to riboflavin, nicotinic acid, pyridoxine, choline and pantothenic acid. For comparison with Text-Figure 1. Legends as in Text-Figure 1.

Thiamine was given every other day. Under urinary thiamine excretion, "first day" refers to the 24-hour period following thiamine administration; "second day" to the succeeding 24-hour period.

Group 2

Pig no. 6-91. This animal was found dead after 37 days on the "thiamine-low" regimen. It had been entirely well and had gained steadily during the experimental period. No studies were made of the pyruvic acid content of the blood.

The heart was dilated and weighed 95 gm. The carcass weighed 20.6 Kg., so that the heart comprised 0.45 per cent of the body weight.

On microscopical study, lesions were found in both auricles but none was present in either ventricle. The right auricle (Fig. 1) was more severely involved than the left. There were numerous scattered necrotic muscle fibers about which leukocytes were gathered. The whole gave the picture of a severe diffuse myocarditis. In the lungs there was fluid in some of the alveoli. The liver was normal. Sections of the nervous tissues, including the brain, showed no lesions.

Pig no. 6-90. This animal died suddenly after receiving the "thiamine-low" diet for 57 days. During the last 10 days of life it vomited several times and began to lose weight. No studies of the pyruvic acid content of the blood were made.

The pig weighed 20.6 Kg. and the heart, which was dilated, weighed 140 gm., being thus 0.67 per cent of the body weight.

Microscopically, there were lesions in both auricles and ventricles. The left auricle was more extensively involved than the right; the same was true of the ventricles. There were focal and confluent diffuse areas of necrosis with cellular infiltration; the latter type of lesion was more prominent, however, especially in the ventricles. The interventricular septum was more severely damaged than the walls of the ventricles. Fat stains revealed foci of fatty infiltration of the muscle fibers in the fresh lesions. The lungs showed edema. The nervous tissues were normal.

Pig no. 6-92. After having received the "thiamine-low" diet for 141 days, this animal was sacrificed. Between the 41st and 91st days of the experiment signs of deficiency, in the form of vomiting and anorexia and elevated pyruvic acid content of the blood, were present. Because of the deaths of the other two animals in this group (nos. 6-90 and 6-91), this pig was given more thiamine than the others. As a result, with the exception of elevated pyruvic acid on a single occasion on the 123rd day of the experiment, no signs of deficiency whatever were detected in this animal during the last 41 days of its life. The animal was killed because of a spreading infection of one hind leg.

The carcass weighed 39.2 Kg. and the heart, 220 gm., being 0.56 per cent of the body weight. Grossly the heart was normal.

On microscopical examination the heart was also entirely normal. There were widespread abscesses in the lungs. The nervous tissues showed no lesions.

Group 3

Pig no. 6-54. This animal died suddenly after having received the desiccated liver supplement for 320 days. For the first 120 days it seemed well. During the final 200 days there were at least six episodes of vomiting, accompanied by loss of weight and elevation of the blood pyruvic acid and interrupted on several occasions by the administration of thiamine. The concentration of pyruvic acid was elevated above normal almost continuously for the last 9 weeks of life.

At autopsy the animal weighed 46 Kg. The heart was tremendously dilated and weighed 350 gm. This represented 0.76 per cent of the body weight.

Microscopically there were widespread lesions of the muscle fibers in both auricles and ventricles, particularly striking in the former. The interstitial tissue was diffusely infiltrated with polymorphonuclear and mononuclear leukocytes. Many muscle fibers were necrotic. In the ventricles there were a few confluent focal areas, but for the most part the change here was diffuse, too. There were numerous foci where the muscle fibers had been replaced by collagenous tissue (Fig. 4). The pulmonary alveoli contained fluid. The liver was normal in appearance. Sections of the nervous tissues, including the brain, were entirely normal.

Pig no. 6-62. This animal was sacrificed after receiving desiccated liver for 239 days. During the midportion of this period vomiting and loss of weight occurred and the blood pyruvic acid was observed to be elevated. The pyruvic acid at first dropped spontaneously to normal, a change which was interpreted as being due to decreased food consumption. Soon the pyruvic acid level rose again, however, and this was accompanied by vomiting and anorexia. Following the daily administration of thiamine the pyruvic acid in the blood fell and remained normal for the last 45 days of life, when the animal was sacrificed.

At autopsy this pig weighed 74 Kg. The heart appeared normal, weighing 270 gm. or 0.36 per cent of the total body weight.

Microscopically no lesions were seen in the auricles or ventricles. The nervous tissue was normal.

Pig no. 6-79. This animal was sacrificed after it had received desiccated liver for 230 days. On the 120th day, although there were no clinical signs, the pyruvic acid level of the blood was found to be elevated. With some decrease in food consumption it dropped, but

later rose again; the animal began to vomit coincidentally with this rise. The pyruvic acid concentration remained elevated or at the upper limit of normal until 30 days before death when thiamine was administered. From this time until death the pyruvic acid content of the blood was normal.

At autopsy the carcass weighed 76.4 Kg. The heart, which was not dilated, weighed 330 gm., being 0.43 per cent of the body weight.

Microscopically the myocardium was entirely normal. The nervous tissue showed no lesions.

Controls

In none of the 45 control animals, all of which received thiamine, were any changes found in the myocardium. Some of these pigs were lacking in vitamins of the B group other than thiamine and grew very poorly, and two were given reduced amounts of food adequately supplemented with vitamins. Thus it is evident that partial inanition is not a cause of the lesions observed.

DISCUSSION

We have observed lesions in the auricular and ventricular musculature of six out of nine pigs receiving a diet adequate in all known respects except for its thiamine content. Five of these six animals received crystalline vitamins other than thiamine as the source of the vitamin B complex, while one was fed desiccated liver known to be poor in thiamine but probably adequate otherwise as a source of the B complex. The lesions consisted of focal or diffuse necrosis of myocardial fibers. Such areas were infiltrated by leukocytes and, in the more chronic cases, were replaced by connective tissue cells so that scars resulted.

In the two animals whose mode of death was observed (nos. 6-53 and 6-77) the clinical picture was that of heart failure with labored breathing and cyanosis, which were made worse by exercise. The four other animals showing lesions in the myocardium died quite unexpectedly during the night. The three pigs failing to show any myocardial lesions had been sacrificed. These animals appeared well at the time they were sacrificed and had been receiving thiamine in doses sufficient to reduce the pyruvic acid of the blood to normal levels for the preceding 30 to 45 days (Table I).

In the animal (no. 6-91) dying very early, after deprivation of thiamine for only 37 days, lesions were found in the auricles but none could be discovered in the ventricles, even after numerous sections were made. In the animals dying after longer periods of deficiency,

TABLE I
Relation of Terminal State of Thiamine-Deficient Animals to Pathological Findings

Experimental group	Pig no.	Duration of experimental deficiency (days)	Time required for onset of first symptoms (days)	Terminal state		Focal necrosis		Lungs
				General condition	Blood pyruvic acid (mg./100 cc.)	Auricles	Ventricles	
Group 1, "thiamine omitted"	6-53	156	72	Cardiac failure	7.4	+	+	Congestion and edema Edema Edema
	6-67	83	74	Sudden death	6.8	?	++	
	6-77	246	74	Death following exercise	5.6	+	++	
Group 2, "low thiamine"	6-90	57	41	Sudden death	3.1	++	+	Edema Edema (Abscesses)
	6-91	37	..	Sudden death		+	o	
	6-92	141	41	Good; sacrificed		o	o	
Group 3, desiccated liver	6-54	320	117	Sudden death	7.3	+	+	Edema o o
	6-62	239	115	Good; sacrificed	3.2	o	o	
	6-79	230	135	Good; sacrificed	2.5	o	o	

lesions were observed in the ventricles as well as in the auricles. In no instances were pathological changes found in the ventricles when they were lacking in the auricles. This suggests that the auricles may be more sensitive to thiamine deficiency than the ventricles. It is of interest in this respect that Muus, Weiss and Hastings,¹⁰ using tissue from thiamine-deficient rats, noted that there was a reduction from normal in the O₂ consumption of the auricular muscle *in vitro* while that of the ventricles remained unchanged. They were unable, however, to find any correlation between the degree of reduction of O₂ consumption of the auricle and the severity of the deficiency.

In two animals in which episodes of thiamine deficiency, marked by anorexia, vomiting and elevation of pyruvic acid in the blood, had been observed at intervals during their course, scars indicative of former necrosis of the myocardium were found. It must be pointed out, however, that in the three pigs sacrificed at a time when they showed no signs of thiamine deficiency, no fresh lesions were observed in the myocardium

and no scars could be found as evidence of earlier episodes of deficiency such as these pigs had suffered. Connective tissue stains were made in addition to hematoxylin and eosin preparations but no indication of scarring could be made out. It would appear, then, that while scars marking the sites of previous necrotic myocardial fibers may be found in some animals, the lack of such scars does not necessarily indicate that thiamine deficiency has not been present.

A constant finding in the pigs showing lesions at autopsy was marked cardiac dilatation. When the size of the hearts of the experimental group was compared with that of controls it was also found that some of the former were enlarged in respect to the body weight. From observations on normally growing animals receiving all known vitamins we have found that the heart represents about 0.30 to 0.40 per cent of the total body weight. In four of the experimental animals the heart weight exceeded this and was as great as 0.76 per cent in one animal. It cannot be concluded, however, that these hearts were hypertrophied. When body growth is impaired, as was the case with the thiamine-deficient pigs, an alteration in the ratio of heart to total body weight occurs. Thus we have observed a heart weight-body weight ratio as high as 1.0 per cent in an animal receiving thiamine as well as riboflavin and nicotinic acid but growing very poorly as the result of deficiency of pantothenic acid and pyridoxine. Loss of body weight without corresponding loss of heart weight may give a false impression of cardiac hypertrophy.

In only a few of the many experiments on vitamin B₁ deficiency reported hitherto have specific lesions of the myocardium, such as those we have noted, been described and in none have they been as extensive as those we have observed. Swank,¹ using a diet partially deficient in thiamine, found myocardial lesions in pigeons that had exhibited gross evidence of cardiac failure. These consisted of scattered foci of necrotic muscle fibers which in two instances were infiltrated with leukocytes. Observations on thiamine-deficient dogs by Swank, Porter and Yeomans² revealed small scattered foci of necrotic muscle fibers in 3 out of 14 instances. Many of these foci were infiltrated with polymorphonuclear leukocytes. One animal showed a "loose scarring of the myocardium." In 7 pigs, on a diet supplemented with sulfite-treated liver and whey, Van Etten, Ellis and Madsen³ found scattered areas of "atrophy and necrosis" of heart muscle fibers which were considered suggestive of infarction. In foxes dying of Chastek paralysis, a disorder which has been attributed to thiamine deficiency, Evans, Carlson and Green¹¹ have described areas of necrosis of myocardial fibers and, in a few instances, proliferation of connective tissue.

In human beriberi, pathological observations have for the most part been limited to gross study of the heart; few detailed microscopical studies have been made. There is controversy whether the chambers of the right side are both hypertrophied and dilated or merely dilated. Vedder¹² stated that "the heart is always considerably enlarged, particularly on the right side." In the majority of the cases studied by Weiss and Wilkins⁴ "the weight of the heart was normal and there was moderate dilatation of the right ventricle." From an analysis of the literature one is struck by the paucity of actual measurements of the heart weight, reliance having been placed on its appearance. Studies of the weights of the individual ventricles are certainly much needed.

It does not appear that sufficient attention has been paid to the histological appearance of the "beriberi heart" to warrant any specific conclusions. Although Wenckebach¹³ and Weiss and Wilkins⁴ have noted "hydropic" degeneration of the myocardial fibers, the latter observers found the same change in other types of heart disease as well. It would be of particular interest in the light of our observations on pigs to study carefully the auricular musculature of patients dying of beriberi.

It should be pointed out that there are a number of reports of "isolated myocarditis" or "Fiedler's myocarditis," all of unknown etiology, which bear a striking similarity to the changes we have encountered in pigs. The possibility that some of these may have been instances of thiamine deficiency warrants consideration.

Cardiac lesions similar to those we have described have been noted in other experimental nutritional deficiencies. Focal necroses of myocardial fibers have been found in ascorbic acid deficiency in the guinea pig.¹⁴ We have recently encountered similar lesions in rats whose dietary potassium had been greatly restricted.¹⁵ In the latter animals, however, the lesions were much more prominent in the ventricles than in the auricles; the rats, furthermore, did not die of cardiac failure. From the standpoint of potassium content the diet employed in the present studies was entirely adequate.

At one time it was suggested that the cardiac manifestations of human beriberi are due to degeneration of the vagus nerves or to respiratory paralysis.¹⁶ These hypotheses are now discredited and our own observations give them no support; the vagus nerves were examined in all the pigs and were found to be normal. As to the "water retention" theory proposed by Wenckebach,¹³ we have already pointed out that hydropic degeneration is found in hearts failing as the result of a variety of causes. It may be added that Wilkins and Cullen¹⁷ found an increase in the water content of the myocardium of both

ventricles in the hearts of patients dying of congestive failure of various types.

Although it is evident that careful histological studies of the hearts of patients dying of beriberi are needed, it is to be expected that in some instances, at least, no lesions will be found. Judging by what is known of the function of thiamine, it is to be expected that a metabolic disorder of the myocardium occurs before anatomical changes take place and that death may ensue before recognizable lesions have developed.

It is of some interest to speculate as to the factors which may have led to the death of our thiamine-deficient animals and to consider the possible reasons for the development of the lesions in the myocardium. The age at which animals are placed on the deficient diet probably influences the interval of time in which the effects of the deficiency make their appearance, since the more rapidly growth is taking place the more thiamine may be needed. The early death of pigs nos. 6-91 and 6-90 is consistent with this view. Another factor, no doubt, is the degree of activity of the animals; a young, lively animal possibly being more likely to develop sudden failure through activity than a less energetic, older pig. In this connection it is of interest that Keefer¹⁸ observed that cardiac failure in cases of beriberi occurred particularly in patients who showed little or no neurological disturbances and were therefore more active, and that Swank, Porter and Yeomans² noted the absence of cardiac failure in dogs which became paralyzed. The amount of food consumed is probably another important factor because with a reduced food intake the requirements for thiamine are lowered. We were able to observe in several animals that the pyruvic acid content of the blood fell when the animals developed anorexia and so consumed less food.

It has been suggested from time to time that pyruvic acid may be toxic and that the accumulation of this substance in the tissues may damage them. Haynes and Weiss¹⁹ administered large amounts of pyruvic acid, sodium pyruvate and related substances to normal and thiamine-deficient rats. Although slight changes in heart rate and in the T waves were noted, they concluded that it is unlikely that the accumulation of metabolites is the important factor in the production of abnormal cardiac manifestations in deficient rats since the amounts necessary to produce changes were very great. Lu²⁰ came to the same conclusions. If pyruvic acid is itself toxic, one wonders whether it is necessary for the pyruvic acid to reach certain levels in the blood and to be maintained at such levels for some time before cardiac lesions will develop. In the experimental studies of Haynes and Weiss and of

Lu no sustained elevation of the blood pyruvic acid was produced. Furthermore, histological studies of the myocardium were not made. Our own data are insufficient to answer these questions.

In view of the rather general conception that thiamine is the "anti-neuritic vitamin," it is worth commenting on the absence of neurologic-al changes, both clinical and pathological, in the pigs we have studied. Recent observations from this laboratory have shown that ataxia, accompanied by sensory neuron degeneration, may be produced in swine when either pantothenic acid or pyridoxine is omitted from the diet.⁷ Up to the present time no investigations have been carried out in other animals fed thiamine-deficient diets supplemented with adequate amounts of the other crystalline vitamins of the B group. The experiments of Swank¹ in pigeons are invalidated because in the main no vitamins of the B group other than thiamine were administered and in other experiments autoclaved diets were fed. It is possible that by autoclaving, other vitamins than thiamine may be damaged.

The negative findings in pigs, together with evidence from the critical analysis of Meiklejohn²¹ concerning the lack of proof of any relationship of thiamine to human polyneuritis, raise doubt in our minds as to the rôle of thiamine deficiency in the production of pathological changes in the nervous system.²² It is quite possible that degenerative changes heretofore attributed to lack of vitamin B₁ may have been due to the lack of other vitamins removed from the diet by autoclaving, or missing as the result of other causes. In the light of the newer information concerning the number of vitamins composing the B complex and their distribution in foods, it can no longer be assumed that the human disorder, beriberi, is caused by the lack of one vitamin only. If we may judge by our studies in pigs,^{6, 7} beriberi develops as the result of deficiency of two and probably three vitamins and no doubt other factors and deficiencies play a part as well.

Lesions similar to those observed in Wernicke's disease have been described in pigeons by Alexander, Pijoan and Myerson²³ and in foxes suffering from Chastek paralysis by Evans, Carlson and Green.¹¹ Both groups of investigators found that the administration of thiamine prevented the development of the lesions. It should be pointed out, however, that a basal diet of polished rice supplemented only with riboflavin and vitamins A, C and D was employed by Alexander, Pijoan and Myerson. The presence of a "severe degree of lipoidosis" in the liver of the foxes studied by Evans, Carlson and Green makes one wonder whether in these experiments also other dietary factors were lacking as well as thiamine. In the present study examination of the brains of three representative animals failed to reveal any lesions.

SUMMARY

1. Cardiac dilatation without hypertrophy, and focal and diffuse myocardial necrosis were the characteristic findings in six pigs dying of thiamine deficiency. In one instance the areas of necrosis were so large that they could be seen with the naked eye. The lesions were infiltrated with polymorphonuclear and mononuclear cells.

2. Areas of necrosis were found only in the auricles in the pig dying after the shortest period of deficiency. In the remaining animals lesions were found both in the auricles and in the ventricles.

3. In two animals which had passed through several episodes of severe thiamine deficiency, scars marking healed necrotic lesions were found.

4. In three experimental animals that were sacrificed at a time when no clinical or chemical evidence of thiamine deficiency was present, no pathological changes were found.

5. No cardiac lesions were observed in a large number of animals dying as the result of other types of vitamin deficiency or in those in which inanition alone was produced.

6. No changes whatever were found in the nervous systems of the thiamine-deficient pigs.

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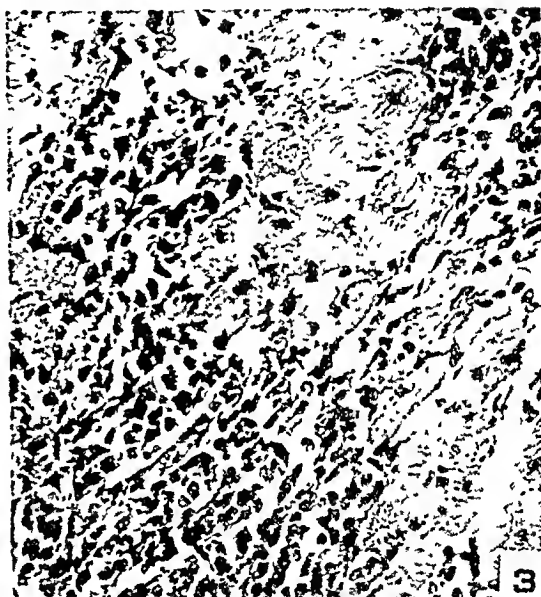
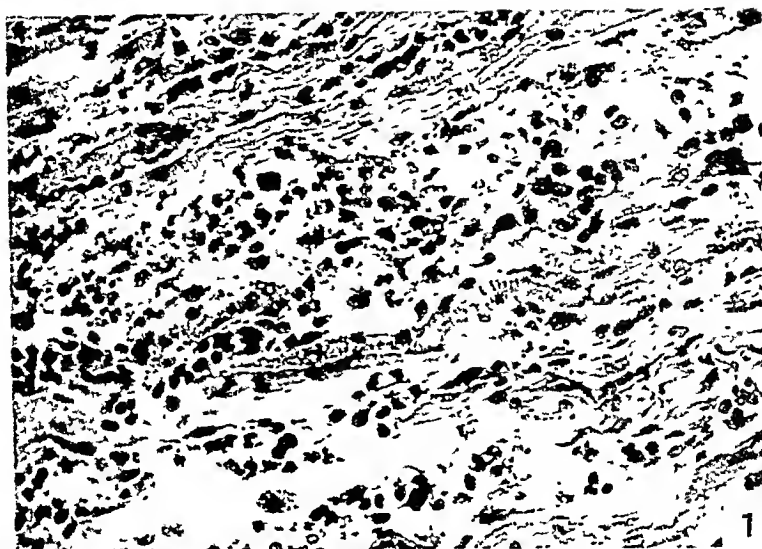
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{ Illustrations follow }

DESCRIPTION OF PLATE

PLATE 40

- FIG. 1. Photomicrograph of right auricle of pig no. 6-91, dying after 37 days on the "thiamine-low" regimen. There is extensive leukocytic infiltration where myocardial fibers have become necrotic. $\times 200$.
- FIG. 2. Photomicrograph of left ventricle of pig no. 6-53 to show edge of large, macroscopical lesion. $\times 90$.
- FIG. 3. Photomicrograph of left ventricle of pig no. 6-53 showing foci of leukocytes, mainly mononuclear, in areas where muscle fibers have disappeared. $\times 200$.
- FIG. 4. Photomicrograph of left ventricle of pig no. 6-54 to show an area where the myocardial fibers have disappeared and have been replaced by collagenous fibers. This is far in excess of the normal amount of connective tissue. Mallory's aniline blue stain. $\times 200$.



Follis, Miller, Wintrobe and Stein

Myocardial Necrosis in Thiamine Deficiency

NEOPLASTIC DISEASE OF THE PANCREAS OF SNAKES (SERPENTES)*

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Some years ago, in the course of a routine autopsy of a pine snake (*Pituophis sayi*), the pancreas attracted attention because of its apparent enlargement. Upon histologic examination, the parenchyma was found to have been largely replaced by tissue which appeared to be carcinoma.¹ Since neoplasms of any sort have rarely been found in reptiles,² this observation led to microscopic examination of the pancreas of each animal of all species of this class of vertebrates that died while on exhibition in the Zoological Garden. As a result, changes which corresponded closely to epithelial neoplasia were found to be comparatively common in the pancreas in several species of snakes, but not in other types of reptiles. Of a series of 73 animals, representing three families of the order Serpentes, 24 had developed lesions of this character, and in 9 of these, the greater part of the gland was replaced by carcinoma.³ Changes in others of this group could be interpreted as developmental stages of the disease, but either were too far advanced to permit an opinion of their origin and development or they were confused by diffuse inflammatory reactions, abscesses and autolysis. Hence, it seemed essential to further study to obtain animals demonstrating earlier and uncomplicated stages of the process.

Members of each of the families that were included in this series had developed this disease, but the majority of the group, and of the animals in which the pancreas was involved, were species of Crotalidae and Colubridae native to North America. The greater number of these had died within 4 or 5 months after capture. It was assumed, therefore, that if numbers of the more susceptible species were killed after 2 or 3 months of captivity, they would provide many examples of developmental stages of the disease.

Choice of species for purchase and slaughter was determined by cost, space requirements and ability of collectors to supply them in quantity, as well as by apparent susceptibility. The ones selected were the prairie rattlesnake (*Crotalus confluentus*), the Jersey pine snake (*Pituophis melanoleucus*) and the black racer (*Coluber constrictor*). These animals were kept in exhibition cages at the Zoo-

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logical Garden until killed. It developed also that the pancreases of the diamond-backed rattlesnakes (*Crotalus adamanteus*), and of water moccasins (*Agkistrodon piscivorus*), apparently the most and the least susceptible of the Crotalidae, might be obtained in fixative from a Florida canning plant which prepares snake meat commercially. These snakes were said to have been killed within a few days after capture.

While these animals were being assembled and studied, microscopic examinations of all other specimens that became available through routine autopsies were continued. In no instance was any change which might be interpreted as a stage of the disease found in the pancreas of reptiles other than snakes. And, contrary to expectations, it was soon evident that the character and extent of lesions to be found in sections were not predictable from gross examination, nor did the frequency of the disease in snakes killed for examination approximate that in those dying spontaneously. Hence, neither biopsy nor transplantation was attempted. Efforts to trace the development of the disease and to determine its nature have been limited, therefore, to interpretation of a variety of changes found upon microscopic study of the pancreas.

THE NORMAL PANCREAS

The pancreas in the several species of snakes included in the present series is a solid body, oval, reniform or angular in outline, enclosed by the mesentery and closely applied to the serosa of the duodenum a few centimeters below the pylorus. Its transverse diameter approximates that of the gut and its length is about twice its width. The common bile ducts (these usually are multiple but within a common sheath) pass through its midportion and enter the intestine close to the pancreatic ducts. In color the organ may vary from dull white to pale pink or brown, and, in consistency, it suggests a lymph node. The spleen, which usually is much smaller than the pancreas, lies on or near the upper pole, or the upper pole may be drawn out into an elongated, narrow structure, as it is in birds, to unite with the spleen, a feature more characteristic of the Boidae, than of other types of snakes in this series. In any event, the spleen and pancreas often are incompletely separated by distinct capsules, and tissues may be mixed about the line of union, a feature which probably explains the occasional presence of neoplastic epithelium within the spleens of certain of the animals of this series.

In the normal pancreas of snakes, cells of the duct system and acini are closely similar to those of mammals and birds but, as a rule, the parenchyma is not subdivided (Fig. 1). Stroma is scanty except at

the periphery and about the larger ducts. Islands of Langerhans usually are composed of eosinophilic, columnar cells, or these cells may contain eosin-staining granules. These bodies commonly are large and irregular. They are found chiefly in the region adjacent to the spleen and occasionally one or more may be seen within the splenic tissue.

Since most of the glands examined were less than 2 cm. in length, complete sections through the long axis, and in a plane parallel with the course of the larger ducts, were easily prepared. These gave maximum areas for study and usually included views of the spleen and of several large pancreatic ducts.

NEOPLASTIC DISEASE OF THE PANCREAS

Changes that have been interpreted as initial phases in the development of epithelial neoplasia were found in four of the snakes that died in captivity, as well as in three *Crotalus confluentus* and three *C. adamanteus* which were killed for examination. The pancreases of these animals contained small scattered foci in which acini were distorted and dilated, and epithelium within them was detached and fragmented or replaced by flattened, indistinctly outlined, pale-staining cells, the nuclei of which were pleomorphic, hyperchromatic and irregularly distributed (Figs. 2 and 3). Of these lesions, those in which epithelial degeneration was the pronounced feature were not well outlined, but within them acini were clearly separated from one another and from surrounding glandular elements as if by local edema (Fig. 2). When glandular epithelium had been replaced by other cells, the focus contained fewer subdivisions, fibrous tissue cells were increased and polymorphonuclear leukocytes and lymphocytelike cells accumulated in small numbers within the involved area and in the surrounding tissues. Often fibrous tissue seemed to outline the area rather sharply.

In only one case did these early focal lesions center about obviously necrotic tissues, and this in an animal dying of acute bacterial infection with many small thrombi in various organs. Thrombi accounted for focal necrosis of the pancreas in this instance. Search for the primary causes of the focal lesions was fruitless in all other animals, however.

Glandular tissue of the pancreases which contained the small focal lesions usually was otherwise unchanged. Normal acini lay close by the degenerating ones. But in these and in all other specimens in which were found lesions that have been interpreted as some stage in the development of epithelial neoplasia, cells of the duct system presented various grades of hyperplasia, usually moderate, sometimes marked, but the various grades were not associated closely with the degree of change in the acini.

Larger focal lesions which could have developed from the smaller, more isolated ones were found more frequently and were associated with more extensive changes of the gland. These occurred in 15 snakes that died spontaneously, as well as in four *Coluber constrictor* that were killed for examination. In addition to increased size of these second stage lesions, abnormality of epithelium within them was more pronounced than in the smaller ones, stroma was increased in and about them and some of them, especially those which formed at the periphery of the pancreas, had the appearance of expanding masses (Figs. 4 and 5). Rather than retaining some suggestion of acinous pattern, epithelial cells were elongated, flattened, indistinctly outlined and arranged into small branching cords and ducts. These lay in loosely arranged masses with scanty fibrous tissue cells between, but fibrous tissue increase was more pronounced about the periphery of lesions, in the remnants of normal acinar tissue, and in the capsule rather than within the lesions themselves. In some instances (Fig. 4), polymorphonuclear leukocytes and large and small lymphoid cells were numerous in and about the lesions, but, as in the examples of earlier stages of the disease, abscesses were not formed.

Glandular tissues about these larger focal lesions usually presented well defined changes. Over rather wide areas acini were dilated and their lumina filled by eosin-staining coagulum. Appearances of glandular epithelium within these areas varied; some acini were well preserved but many were undergoing degeneration. However, there was nothing to indicate that these larger foci had expanded by transformation of the altered units into the ducts and cords mentioned earlier. Rather, they seemed to be degenerating as the new-formed structures enlarged. But in spite of these relatively extensive focal changes, considerable amounts of unaltered glandular tissues usually remained at this stage of the disease.

Many, if not all, of the examples of more advanced forms of this disease may be interpreted as having been formed by continued growth of the centers of abnormal regeneration in one or in several parts of the gland. In 16 animals, lesions were considerably more extensive than those of the second group but, still, islands of comparatively normal tissue were present. These cases have been classed as a third stage of the disease. All of these animals died while on exhibition. In them, epithelial elements of the focal lesions assumed a more definite organization, forming compact masses of irregular tubules, or apparently expanding adenomata (Figs. 6 and 7). Their component cells were clearly defined and pleomorphic, with dense cytoplasm and large, irregular, vesicular nuclei, and the cell pattern more closely resembled

that characteristic of carcinoma. These lesions seemed to be expanding, and compressing surrounding tissues rather than being separated from them by edema. Stroma within them was increased and compact. In the remainder of the gland, stroma was also increased and the fibers separated. Leukocytes and lymphoid cells likewise were present in increased numbers, and many large phagocytic cells (epithelioid cells) were noted.

Intervening glandular tissue retained a normal pattern fairly well, sometimes with considerable degeneration of the epithelium and dilatation of acini. As a rule, it amounted to less than one-half of the organ. In these cases, as well as in those exhibiting more advanced changes, islet tissue could not be identified.

Finally, a group of ten animals, all of which died in captivity, supplied the specimens in which lesions were of a type that suggested carcinoma most strongly. In so far as could be determined by sections through the long axis of the gland, neither acinar nor islet cells remained in recognizable form. Microscopic appearances of these specimens varied. Three were largely fibrous tissue with islands of epithelium forming adenomatoid structures. In the remainder, fibrous tissue was less abundant, and epithelial elements more closely placed but forming, nevertheless, equally abnormal structures (Figs. 8 and 9). These variations were no greater than might be expected in any group of neoplasms of glandular tissues. In three animals of this group the capsular tissues contained small adenomatous growths which suggested local extension, and, in two, similar structures were present in the wall of the gut adjacent to the pancreas, which probably did not indicate local invasion, but rather that the displaced tissue responded as a unit of the organ. Those specimens of this group which were largely scar tissue probably resulted either from local infection and abscess formation, or from unexplained degeneration of the abnormal glandular tissue. There was evidence that both of these processes were operating.

Species Involved and Frequency of Disease

Failure to list the species of snakes that died spontaneously and in which this disease of the pancreas occurred is intentional. There seemed to be no point in this, since no one variety predominated in any particular subgroup. Instead, the entire series has been tabulated to show the families in which the disease occurred and the number of specimens of each that were examined. Two of the families, Crotalidae and Colubridae, have been subdivided to genera and species because they contained the bulk of the series and among them there apparently

were differences in susceptibility. The pancreases of reptiles of many other varieties were examined, but only those families in which the disease occurred have been included in the following table.

Table I contains records of 397 specimens, of which 136 died while on exhibition in the Zoological Garden. These animals were members of five families of the order Serpentes: Crotalidae, Colubridae, Boidae, Elapidae and Viperidae. Column 1 of the table contains the number of animals in each of these groups, and the second column shows the

TABLE I

Number and Variety of Snakes Examined and Frequency in Each Group of Pancreatic Lesion of a Type that Suggested Epithelial Neoplasia

Families	Died		Killed	
	Number examined	Number positive	Number examined	Number positive
CROTALIDAE	92	33	184	6
<i>Crotalus adamanteus</i>	25	15	56	3
<i>Crotalus confluentus</i>	20	3	115	3
<i>Crotalus horridus</i>	17	6	0	0
<i>Crotalus ruber</i>	11	7	0	0
<i>Sistrurus miliaris</i>	5	1	0	0
<i>Agkistrodon mokasen</i>	4	1	0	0
<i>Agkistrodon piscivorus</i>	10	0	13	0
COLUBRIDAE	30	6	77	4
<i>Pituophis melanoleucus</i>	7	1	24	0
<i>Pituophis sayi</i>	4	1	0	0
<i>Coluber constrictor</i>	3	2	53	4
<i>Lampropeltis getulus</i>	2	2	0	0
<i>Natrix sipedon</i>	14	0	0	0
BOIDAE	5	3		
ELAPIDAE	4	2		
VIPERIDAE	5	1		
Total	136	45	261	10

number of cases in which there was some grade of pancreatic disease which might be interpreted as part of the process of epithelial neoplasia. This part of the series supplied 45 of the positive cases, which is to be contrasted with 7 cases among 195 snakes that were killed for examination. It will be recalled that, of the 261 specimens listed in column 3 of the table, 56 *Crotalus adamanteus* and 13 *Agkistrodon piscivorus* were killed at the canning plant. Thus the 10 positive cases among snakes killed for examination contain 3 that were not subjected to the influence of captivity.

The number of representatives of any one of the species of Crotalidae and Colubridae listed in the table is rather too small for satisfactory comparison of incidence, but apparent differences in susceptibility may be noted. In particular, of 25 specimens of *Crotalus adamanteus*, 15 were involved, while none of 10 *Agkistrodon piscivorus*

developed lesions of the pancreas. Among the Colubridae, the contrast is not so sharp, but 6 of 16 snakes of four species developed the disease and none of 14 *Natrix sipedon* was involved. In general, failure to develop this condition has been paralleled by the ease with which animals of various species become adjusted to captivity.

DISCUSSION

Obviously the title of this paper is not completely justified except by the 10 cases which have been designated as stage four in the development of this disease of the snake's pancreas. Whether or not these specimens may be regarded as true neoplasms seems to depend largely upon definition. Certainly their morphology appears to warrant this classification, although metastases were not found and local extension occurred infrequently. Perhaps the absence of metastases may be accounted for by the small number of cases in the series and does not reflect the growth characters of the abnormal tissues. Lucké examined a much larger series of carcinomata of the kidney of *Rana pipiens* before finding secondary growths² and observed that, in comparison with similar tumors of man, this neoplasm of frogs metastasized later in the course of its development.⁴ Moreover, the lesions of snake's pancreas affect the whole organ, replacing acinar and islet tissues completely. Thus, possibly, interference with pancreatic function ends life before growth characters of the new tissue may be fully expressed.

Again there can be no certainty that the focal lesions are phases in the development of the more extensive changes; but if one bases judgment upon morphology only, this seems to be a reasonable assumption. Granting it to be true, then the various stages of the process are examples of regeneration and hyperplasia which occasionally undergo neoplastic change but which more frequently are found in intermediate states. For it seems clear that the initial stages of the disease are simply irregular focal degeneration of acinar epithelium followed by replacement of these cells by ones which resemble those of the terminal ducts, which is entirely in keeping with experimental studies of regeneration of pancreatic tissue of mammals.⁵ But this disease of the snake's pancreas is unique, not so much because of its frequency, as because it emphasizes the possible difference in susceptibility to neoplasia inherent in the organs and tissues of various animal types.

Search for a cause or causes of the primary foci of degeneration has been fruitless. Blockage of ducts by inflammatory changes has not been definitely established in any of the animals. Acute inflammatory disease of the intestine which might produce such an effect often was

present in the animals that died spontaneously, but not always so. And this factor could be ruled out completely in the animals that were killed for examination. Parasitic organisms, especially species of Sporozoa, which might stimulate hyperplasia of ductal epithelium, were found only in a few snakes, none of which have been included in the present series since changes associated with these organisms were distinctly different.

SUMMARY

The acinar tissue of the pancreas in many species of snakes undergoes unexplained focal necrosis followed by abortive regenerative growth, apparently of the terminal ducts, producing small, edematous adenomalike structures. These areas presumably enlarge, and with their enlargement, leukocytes infiltrate the organ, fibrous tissue is increased and there is further degeneration of acinar and islet epithelium, until occasionally the whole organ is replaced by tissue which has the histologic characters of carcinoma. In a series of 136 snakes of five families of the order Serpentes, all of which died in captivity, 45 presented some stage in the development of this disease. But of 261 snakes of species that seemed most susceptible to the disease, killed for examination 60 to 90 days after capture, only 10 had developed lesions of this sort and none of them presented the more advanced stages of the disease.

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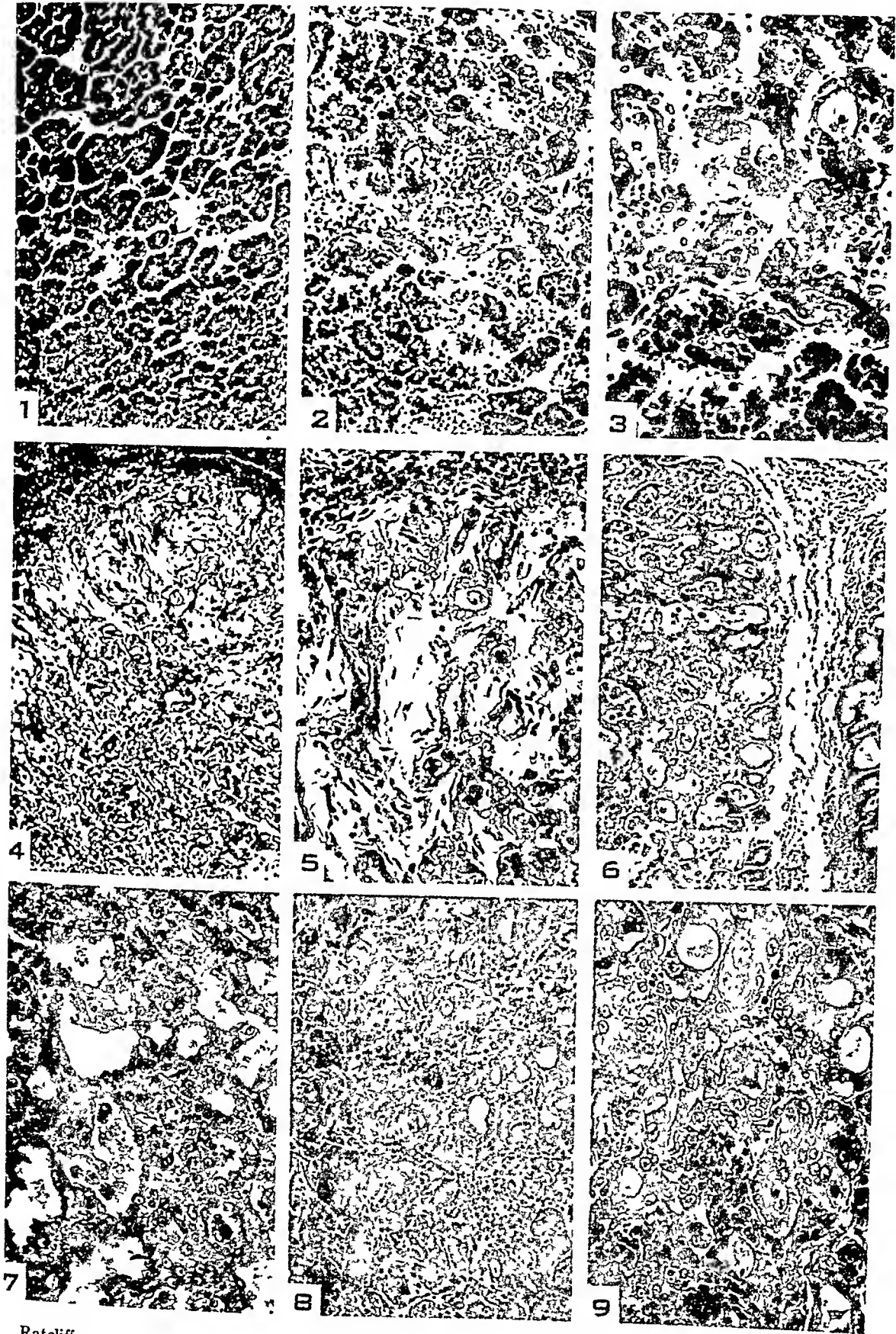
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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 41

- FIG. 1. Normal pancreatic tissue from a black racer (*Coluber constrictor*), showing the arrangement of acini. $\times 90$.
- FIG. 2. A first stage lesion of the pancreas of a black racer (*Coluber constrictor*), dying 3 months after capture. This area shows unchanged acini about acini that are undergoing degeneration and being replaced by epithelium which probably grew in from the terminal ducts, local edema and accumulation of phagocytic cells. $\times 90$.
- FIG. 3. Central part of Figure 1. $\times 200$.
- FIG. 4. Second stage lesion of the pancreas of a diamond-backed rattlesnake (*Crotalus adamanteus*), dying after 40 days in captivity. This had formed adjacent to the spleen, and acinar tissue has been replaced by thin-walled tubules and loosely arranged connective tissue. $\times 90$.
- FIG. 5. The outer margin of a second stage lesion adjacent to that shown in Figure 4, showing delicate branching tubules which extend to the margin of splenic tissue. $\times 200$.
- FIG. 6. Third stage lesion of the pancreas of a timber rattlesnake (*Crotalus horridus*). Two adenomatous lesions may be seen, separated by fibrous tissue which passes in from the capsule. $\times 90$.
- FIG. 7. Left central part of tissue shown in Figure 6. $\times 200$.
- FIG. 8. Fourth stage lesion of the pancreas of a black racer (*Coluber constrictor*). This field is representative of the entire organ. No trace of acini or islet cells was seen. $\times 90$.
- FIG. 9. Cellular character of a part of the field shown in Figure 8. $\times 200$.



Ratcliffe

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INFLAMMATION IN EMBRYONIC LIFE

I. CHANGES PRODUCED BY PARTICULATE MATTER AND BY A CHEMICAL AGENT *

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Although a great variety of infectious agents have been grown upon the membranes of chick embryos, little detailed information concerning the character of inflammation in embryonic life has been obtained. In the present study changes following the introduction of particulate matter and of a chemical irritant have been observed at different stages of development.

Bauer¹ applied different agents, including benzine, a mixture of benzine and paraffin, aniline, and aluminum powder, to the outer surface of the chorion by introducing it into the air space of the shell. These substances caused widely distributed rarefaction of the mesenchymal tissue in some places and hyperplasia elsewhere; formation of blood vessels and hemopoiesis were more active than usual. Local changes produced by these agents were not studied. Proliferative changes in the three layers of the chorioallantoic membrane following removal of a part of the eggshell have been described by Goldsworthy and Moppett.²

When Schneider³ injected carbon particles of India ink in large quantity into the vitelline vein of chick embryos from 2 to 14 days old, they were found in endothelial cells in all parts of the embryo and its membranes, but when the quantity injected was diminished, endothelium of blood vessels of the area vasculosa, of the liver and of the glomeruli took up carbon particles whereas other endothelial cells contained little or none. At this stage of development no difference between Kupffer cells and other lining cells of the capillaries of the liver was evident.

The chorioallantoic membrane has been used by Goodpasture, Woodruff and Buddingh⁴ for the cultivation of many filterable viruses.

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Woodruff and Goodpasture⁵ infected with fowl pox the chorioallantoic membrane and embryonic skin of chick embryos at an early stage of development and found proliferation of ectodermal and of entodermal cells with inclusion bodies in both. Infection of chick embryos, 12 to 19 days old, with Rocky Mountain spotted fever by Lillie⁶ caused infiltration of the membrane with cells resembling lymphocytes and proliferation of fibroblasts about blood vessels.

Cultures of *Staphylococcus aureus* and of hemolytic streptococci applied by Goodpasture and Anderson⁷ to the chorioallantoic membrane of chick embryos from 6 to 14 days old caused superficial necrosis but these microorganisms did not invade the tissues of the membrane. Diphtheria bacilli grew upon the surface and apparently killed the embryo by their toxin. Typhoid bacilli entered the ectodermal cells of the membrane. *Brucella abortus* and the avian tubercle bacillus entered ectodermal and entodermal cells and, penetrating into the mesoderm, were found in fibroblasts and mononuclear phagocytes. Both epithelial and mesodermal cells were favorable sites for the multiplication of these bacteria.

Meningococci inoculated by Buddingh and Polk⁸ upon the chorioallantoic membrane of chick embryos 12 days old invaded the blood vessels of the membrane and, widely distributed by the blood, found lodgment in the meninges, in the glomeruli of the kidneys and elsewhere. In the meninges they produced meningitis. In embryos 15 days old the microorganism proliferated more slowly, there was greater accumulation of polymorphonuclear leukocytes and no lesions of internal organs were found. Gonococci similarly introduced caused, Bang⁹ found, clouding and ulceration of the membrane with local accumulation of polymorphonuclear leukocytes, but no invasion of internal organs.

The developing embryo was used by Rous and Murphy¹⁰ for the study of tumor implantation. Murphy¹¹ showed that sarcoma and mammalian embryonic tissue implanted upon the membrane of chick embryos grew actively, whereas they failed to grow when grafted into an adult fowl. If the foreign tissue were implanted on the 19th or 20th day of incubation, that is, shortly before hatching, growth failed to occur, and if the tissue had already been implanted in the embryo, growth ceased at the same period of development, the graft being destroyed. In both cases active new formation of fibrous tissue occurred about the graft, and it was invaded and replaced. Danchakoff,¹² Minoura¹³ and Huxley and Murray¹⁴ have observed proliferative changes with cornification in the ectoderm of the chorioallantois produced by the presence of implanted tissue.

METHODS

Chick embryos ranging in age from 36 hours up to the period of hatching received injections of India ink diluted to five times its volume with salt solution, or of turpentine which had been mixed with India ink to mark the site of injection.

Embryos younger than 12 days were injected through capillary pipettes directed by a micromanipulator and older embryos with a tuberculin syringe and fine needle. The shell at the summit of the large end of the egg, taken temporarily from the incubator, was removed with sterile instruments and the shell membrane was then torn away with fine forceps. When embryos were younger than 3 days, from 8 to 12 cc. of albumen were removed with a pipette so that the embryo resting on the top of the yolk could be reached more readily. The opening was covered with part of the shell of another egg and returned to the incubator.

Injections into the bodies of young embryos were made near the tail because those elsewhere have often caused death of the embryo. With older embryos it was possible by gentle manipulation to expose for injection a leg or wing. After different intervals following the injection of an irritant, embryos were removed from the egg and fixed in Bouin's solution. Eggs were opened but left uninjured otherwise, and were examined after incubation as controls. Small embryos or the injured parts of larger embryos were sectioned serially. Sections were stained with a variety of methods but the best results were obtained with hematoxylin and eosin-azure and by examination under the oil immersion lens.

INFLAMMATION CAUSED BY CARBON PARTICLES

Changes in the Membranes

When a suspension of carbon was injected into the amniotic cavities of embryos 3 to 5 days old, usually with injury to the area pellucida close to the body of the embryo by the injecting instrument, the most conspicuous response was *accelerated proliferation of cells* at and near the site of injury. In the chorion and amnion there was hyperplasia of ectoderm and mesoderm and in some instances it had closed the wound. The hyperplastic ectoderm formed multiple superimposed cells, and beginning abnormal keratinization was shown by the deep eosin stain of the superficial cells (embryo no. 138, 4¾ days old, and examined 18 hours after injury). About a small collection of carbon particles proliferating ectodermal cells of the amnion formed a rounded projecting nodule.

The underlying mesodermal cells underwent similar multiplication,

and mitoses were numerous. When injected carbon particles accumulated in contact with the wall of a small blood vessel, proliferation of endothelial cells occurred and was limited to the part of the endothelium adjacent to the carbon so that a small mass of cells projected into the lumen (no. 39, $3\frac{1}{2}$ days old, examined after 24 hours).

In an embryo (no. 37) injected when $3\frac{1}{2}$ days old and fixed 10 hours later, numerous *mononuclear phagocytes* containing carbon particles were found in the cavity between chorion and amnion and in contact with the inner surface of the former. Some of the cells that ingested carbon contained erythrocytes as well, and intracellular digestion of them was evidently in progress, for nucleated red cells were found in different stages of disintegration.

In the splanchnopleure in contact with the yolk of this embryo, *granulocytes* were readily found in small groups, their characteristic acidophilic granules being stained with eosin. Most of these cells had indented nuclei and cytoplasm closely packed with granules, but in some instances similar granules appeared in the basophilic cytoplasm of mononuclear cells that were still recognizable as hemocytoblasts. This formation of granulocytes occurred in membranes of normal embryos of the same age and evidently was not caused by the presence of carbon. No granulocytes were found in the membrane adjacent to collections of injected carbon particles.

Changes in the Body of the Embryo

When carbon was injected into the tissue of an early embryo (no. 35 A, $3\frac{1}{2}$ days old) *accelerated proliferation of cells* was evident at the site of the injury 3 hours after the injection. With superficial destruction of the ectoderm there was sometimes active proliferation of the adjacent cells of the mesoderm and a papillary mass formed by them projected from the wounded surface. These mesodermal cells had a rounded form and mitotic figures were numerous. When the injecting tip of the micropipette passed into the body cavity of an embryo of the same age (no. 36) and injured the mesothelium and underlying tissue adjacent to the bulbus arteriosus, proliferation of mesothelial and mesenchymal cells after 8 hours formed a small mass projecting into the body cavity. Carbon particles within this mass were found in mononuclear phagocytes.

When carbon was injected directly into the mesoderm, the embryonic connective tissue was spread apart by the injected material and erythrocytes were in places abundant, but even after 8 hours there was scant if any cellular reaction. Carbon particles were seen 3 hours after injection (no. 25), attached to the surfaces of the mesodermal cells and their processes and within their cytoplasm.

Mononuclear phagocytes containing carbon particles were found in embryos $3\frac{1}{2}$ days old at the time of injury and fixed 8 hours (no. 35) and 24 hours (no. 36) later. They were found within masses of proliferating mesodermal cells. After 8 hours some of them had reached considerable size and contained vacuoles.

In embryos $3\frac{1}{2}$ or $4\frac{3}{4}$ days old, examined 3, 8, 18 and 24 hours after injury caused by injection of India ink into the mesoderm of the chorion, no granulocytes were found. In embryos (nos. 40 and 41) 8 days old at the time of injection and examined 8 hours later, numerous *granulocytes* with characteristic eosinophilic granules and lobed nuclei were present about the injected carbon. Small round cells with a round nucleus and basophilic cytoplasm were present in the mesoderm adjacent to the injected carbon and in places had collected to form groups. In some of these cells, usually about small blood vessels, eosinophilic granules appeared in the basophilic cytoplasm (granuloblasts). As the granules increased in number, the cytoplasm lost its basophilic stain and various transitions between granuloblasts and granulocytes with bilobed or trilobed nuclei were found. The presence of carbon in the subcutaneous mesoderm had stimulated the new formation of granulocytes.

In one instance (no. 41, 8 days old and examined 8 hours after injection), India ink was introduced into the mesodermal tissues of the leg, and in the perichondrium on the side next to the injury there was an almost continuous row of granulocytes with eosinophilic granules. These cells occurred among the polygonal cells with basophilic cytoplasm that surrounded the cartilage. Granulocytes were abundant in the tissue between the site of injury and the perichondrium.

In older embryos (nos. 33 and 79), 12 and 17 days old at the time of injection, a few granulocytes were found about injected carbon after 3 hours. It is noteworthy that at this period of development granulocytes were readily found in the bone marrow and were present in small number in blood vessels but no evidence that they had migrated from blood vessels at the site of injury was obtained.

In an embryo (no. 94) 19 days old, an *inflammatory reaction similar to that of the adult* was in progress 10 hours after the injection of a considerable quantity of carbon. Some hemorrhage had occurred as the result of injury at the site of injection. Granulocytes in great number accumulated in and about clumps of carbon particles and were found in small number in the surrounding tissue. Here they were numerous within small veins and capillaries. They were often adherent to the endothelium, and granulocytes fixed in process of migration through the vessel wall were readily found. Granulocytes about the carbon had occasionally ingested a few particles. Mononuclear phagocytes were

present in small number, and these were laden with ingested carbon particles.

INFLAMMATION CAUSED BY TURPENTINE AND CARBON PARTICLES

When colorless material was injected into the body of an embryo it was often not possible to discover the site of injection after fixation, but when the material was mixed with carbon particles the site of injury was readily identified. Turpentine was diluted with an equal volume of olive oil and to the mixture was added approximately one-third of its volume of India ink. In two experiments (nos. 106 and 107) the mixture of turpentine, oil and India ink was allowed to dry and form a semisolid mass before it was applied to the site of a puncture into the embryo.

Changes in the Membranes

In one embryo (no. 106, 36 hours old when injected, and examined 11 hours later), carbon was found in and below the ectoderm of the amnion at its junction with the body of the embryo. Endothelial cells in the walls of small blood vessels in contact with clumps of carbon particles had undergone proliferation, which was limited to the part of the endothelial lining adjacent to the carbon. The endothelium was stimulated to form masses of cells projecting into the lumen of the vessel. These cells had round nuclei and basophilic cytoplasm and resembled hemocytoblasts.

In some instances (e.g., no. 124, 3 days old when injected and examined 18 hours later) cells proliferating about clumps of carbon particles within the amnion have carried them to the interior of the cavity and thus brought about their elimination from the tissues of the embryo.

Changes in the Body of the Embryo

Below the surface of an embryo (no. 110) 48 hours old, examined 36 hours after injury, mesodermal tissue had undergone necrosis in a small focus in contact with the neural tube and here the overlying ectoderm and mesoderm had proliferated actively. *Accelerated proliferation* of ectoderm formed projecting papillae within which were keratinizing cells. Multiplication of mesodermal cells was accompanied by abundant new formation of intercellular fibers.

In an embryo (no. 123) 3 days old and killed 12 hours after injection, there was injury of mesodermal tissue below the dorsal surface of the embryo in contact with the neural tube, and here proliferation of fixed mesenchymal cells had occurred. Small round cells with basophilic cytoplasm and round vesicular nucleus infiltrated the injured tissue. These cells resembled hemocytoblasts on the one hand and the

macrophages present in the tissue on the other. These macrophages, which had a rounded outline, a single round nucleus and basophilic cytoplasm, contained nuclear fragments, refractive particles that stained with eosin and occasionally an ingested cell still intact.

In an embryo (no. 113) 12 days old and fixed 9 hours after injury, a few *mononuclear phagocytes* with characters similar to those just described contained carbon particles. In an embryo (no. 146) of the same age, but killed 24 hours after injection of the irritant, mononuclear phagocytes that had ingested carbon particles, red corpuscles and occasionally granulocytes were numerous and in some instances of large size. The origin of these phagocytes from cells with the characters of hemocytoblasts, which multiplied with mitosis and were situated just outside of small blood vessels, was readily traced. At first a small cell with round nucleus and basophilic cytoplasm had ingested a single erythrocyte. Cells with abundant ingested contents became larger, and often irregular in outline. As these cells increased in size, the basophilia of their cytoplasm was lost. It is noteworthy that similar transformation of hemocytoblasts into macrophages was seen within blood vessels of an embryo in which carbon had entered the blood stream and was widely distributed within the blood vessels.

Granulocytes have little part in the reaction that follows the injection of turpentine and carbon particles into early chick embryos. In embryos (nos. 106 and 107), 36 hours old when they received turpentine and carbon, and killed 11 hours later, there was active proliferation of mesodermal cells but no granulocytes were found. In an embryo (no. 123) 3 days old and killed 12 hours after injection, ectoderm and underlying mesoderm on the dorsal aspect of the embryo together with a small part of the neural tube were destroyed, but no granulocytes were found in or about the injured tissue.

In an embryo (no. 103) 6½ days old, and fixed 7½ hours after injection, there was no accumulation of granulocytes at the site of injury, but in an embryo (no. 112) of the same age, fixed 24 hours after injection, injury to the leg had destroyed ectoderm and underlying mesoderm, and there was hemorrhage in places. No carbon particles were found in the injured tissue. Granulocytes were fairly abundant but were limited to an area between the site of injury and the perichondrium of the rudimentary bone. Granulocytes, usually with lobed nuclei, were found here about small blood vessels. A few granulocytes were seen within the lumen of a small blood vessel and in several instances were fixed in the vessel wall itself, as though in process of migration. The number of granulocytes diminished as the actual site of injury was approached, and here none was found.

In an embryo (no. 146) 12 days old, fixed 24 hours after injection,

the site of injury was marked by carbon particles widely distributed and by hemorrhage of small extent. Granulocytes had accumulated and mononuclear phagocytes ingesting carbon particles were numerous. Within the wall and just outside of small blood vessels basophilic cells were in process of transformation into granulocytes, acidophilic granules being sparsely scattered in the basophilic cytoplasm of small cells with round nuclei (granuloblasts). The smallest cells that contained acidophilic granules had a diameter only slightly greater than the long diameter of an erythrocyte; the nucleus was round, and the cytoplasm conspicuously basophilic. No granulocytes were seen within the lumina of blood vessels adjacent to the injury, although granulocytes were abundant in well developed bone marrow in a bone of the leg.

In embryos 17 days old *inflammation similar to that in the adult* was produced by turpentine and carbon. Within 2 hours after injection of the irritant (no. 84), granulocytes collected about carbon particles and were migrating from blood vessels. In the adjacent tissue granulocytes with lobed nuclei were found in abundance within the lumina of small blood vessels. Others were fixed in transit through the wall. After 4 hours (no. 85) similar changes were seen, but after 6 hours (no. 80) accumulation of granulocytes about carbon particles was much more advanced. The granulocytes had taken up a few carbon particles, whereas macrophages adjacent to them were filled with carbon. Some local formation of granulocytes was in progress about small blood vessels, acidophilic granules being found in cells with round or indented vesicular nuclei and basophilic cytoplasm (granuloblasts). Where carbon particles had penetrated close to the periosteum of a leg bone, granulocytes with lobed nuclei were present in abundance in the periosteum and were found within the layer of osteoblasts next to the bone, but in the periosteum distant from the site of injury no granulocytes were found. In embryos (nos. 81 and 82) 17 days old and killed 10 hours after injury, granulocytes in the inflamed tissue were in great part mature with lobed nuclei, but some large granulocytes had a round nucleus and resembled myelocytes. In these embryos phagocytosis of granulocytes by macrophages was conspicuous and after 24 hours (no. 89) phagocytosis of granulocytes and of erythrocytes by macrophages was more advanced.

In an embryo 17 days old macrophages that had taken up carbon particles were found 6 hours after injection of turpentine and carbon (no. 80). After 10 hours (no. 81) mononuclear wandering cells with basophilic cytoplasm appeared in considerable number about blood vessels at the site of injury, and, as they became larger, vacuoles appeared in their cytoplasm. These cells took up carbon particles. Af-

ter 24 hours following the injection (no. 89), mononuclear phagocytes were very numerous and had ingested a large part of the carbon in the tissue. Cells that had taken up carbon contained some granulocytes and erythrocytes as well. Multiplication of mononuclear cells by mitosis had occurred about blood vessels, and from the blood vessel outward the transformation of these cells into macrophages was evident. They increased in size, lost their basophilic stain, became vacuolated, and ingested carbon particles.

DISCUSSION

In embryos from 3 to 5 days old, traumatic injury accompanied by the introduction of particulate matter, namely, carbon, or of an inflammatory irritant such as turpentine causes accelerated proliferation of cells adjacent to the injury, both in the embryo itself and in its membranes. Similar changes are produced by carbon particles (India ink) alone and by turpentine with carbon, but they proceed more rapidly with the latter. Proliferation of ectodermal cells may cause the formation of papilla-like projections, with abnormal keratinization of cells. When ectoderm is destroyed, proliferation of mesodermal cells may produce small masses of tissue projecting above the surface. Carbon introduced by injection may be carried upward by proliferation of ectodermal or mesodermal cells below it and finally may be discharged upon the surface of the embryo. When an irritant is injected into the chorioallantoic cavity or into the body cavity, injury of mesothelium may be followed by proliferation of adjacent mesodermal cells to form a little mass projecting into the cavity. Carbon particles introduced into the tissue may be carried into the cavity by proliferation and desquamation of cells.

With carbon particles alone and with turpentine mixed with carbon particles, small masses of carbon have in some instances lodged just outside of a small blood vessel of the splanchnopleure next to the yolk and here the endothelium has been stimulated to form a small rounded mass projecting into the lumen. This little accumulation of proliferating cells is found next to the carbon particles but none is seen elsewhere.

Carbon particles introduced into the mesoderm of embryos 3 to 4 days old stick to the surface of mesodermal cells and their processes and they may enter the cytoplasm of cells. Save for accelerated proliferation of cells adjacent to the site of injury there is in these early embryos during several hours after injection of carbon particles little cellular reaction. Nevertheless, after 8 hours carbon particles are found ingested by round mononuclear cells that are vacuolated and resemble

histiocytes. The formation of macrophages has been observed in young embryos injected with turpentine and carbon particles. After 12 and 24 hours cells containing carbon particles are fairly numerous and like the macrophages of postembryonic life may contain simultaneously carbon particles, red blood corpuscles and occasionally granulocytes. They develop from small cells with round vesicular nuclei and basophilic cytoplasm not distinguishable from the hemocytoblasts that produce erythrocytes on the one hand or granulocytes on the other. The smallest of these cells are found about blood vessels and those that have ingested a single erythrocyte are readily identified by their basophilic cytoplasm. As they increase in size, the basophilic character of the cytoplasm disappears, vacuoles are abundant in them and the nucleus becomes oval or indented.

Formation of granulocytes from cells with a round vesicular nucleus and basophilic cytoplasm takes place normally in the chorionic membrane on the third day of development and mature granulocytes then enter the circulating blood (Sabin¹⁵). Formation of granulocytes in the bone marrow begins about the ninth day (Danchakoff¹⁶). In the rabbit, Sabin, Miller, Smithburn, Thomas and Hummel¹⁷ have found that the number of leukocytes in the circulating blood is small throughout embryonic life, being about 900 before birth. Immediately after birth the number is approximately 2000, the increase affecting almost wholly the granulocytes.

A few round acidophilic granules make their appearance in the basophilic cytoplasm of extravascular cells which are not distinguishable from those intravascular cells that produce erythrocytes. These granuloblasts contain at first a few round granules which take a dull red stain with eosin and vary considerably in size. The granules later stain deeply, become elongated and fill the cell uniformly. The cytoplasm loses its affinity for basic dyes and the nucleus is round, oval, or indented and vesicular; these cells are myelocytes. More mature granulocytes have a horseshoe-shaped or lobed nucleus and are smaller than myelocytes. Both granuloblasts and myelocytes undergo division by mitosis.

In early embryos no granulocytes are found at the site of injury from 3 to 24 hours after injection of the irritant. The earliest embryo in which granulocytes have been found adjacent to the site of injury has been 6½ days old. Here none has been found at the actual site of injury in the leg, but they have been fairly numerous in a limited area between the injured tissue and the perichondrium surrounding the cartilage which at this period is the precursor of a bone of the leg.

In embryos 8 days old and in older embryos granulocytes have been found in moderate number about injected carbon after about 8 hours.

It is evident that they have been formed locally, presumably by stimulation of cells which can be transformed into them. Adjacent to the site of inflammation, granuloblasts in process of formation from cells with the characters of hemocytoblasts are found, singly and in groups, about small blood vessels. The smallest granuloblasts are round with a round vesicular nucleus and a variable number of clearly defined acidophilic granules within their basophilic cytoplasm. As acidophilic granules become more numerous the basophilia of the cell is lost and various transitions are found between cells with round or oval vesicular nuclei like those of myelocytes and granulocytes with characteristic polymorphous nuclei.

This new formation of granulocytes is limited to the site of inflammation and here cells with the morphological characters of hemocytoblasts found immediately about blood vessels are susceptible of transformation into granulocytes in response to the stimulus consequent upon the presence of an inflammatory irritant. It is noteworthy that cells with the same morphological characters may under other conditions be transformed into erythrocytes or into histiocytes, the former being formed within blood vessels and the latter, usually at least, extravascularly. Moreover, it is probable that under appropriate stimulus hemocytoblasts may produce granuloblasts within the lumina of blood vessels.

In certain tissues cells susceptible of transformation into granulocytes are more numerous than elsewhere. When inflammation has occurred in the neighborhood of the cartilage that is the precursor of long bones, granulocytes have been found in unusually large number in and about the perichondrium. It is possible that cells with the potentiality of forming the granuloblast of the bone marrow are numerous here and undergo prompt transformation into granulocytes under the stimulus of the inflammatory reaction.

In embryos from 17 to 19 days old, that is, shortly before hatching, inflammation acquires the characteristics of postembryonic life. Within 2 hours after injection of an irritant mature granulocytes have collected in considerable number about carbon in the tissue, are present within the lumina of small blood vessels and are fixed in the walls of vessels, presumably in passage through them. After 4 to 6 hours these leukocytes are more abundant and have ingested a few carbon particles, but at this time phagocytosis by macrophages is much more active. Nevertheless, formation of granulocytes in the tissue adjacent to the site of inflammation occurs as in younger embryos, for cells with a round nucleus, basophilic cytoplasm, and a few acidophilic granules are found about small blood vessels.

SUMMARY AND CONCLUSIONS

In embryos 3 to 5 days old, accelerated proliferation of cells is the most conspicuous reaction to injury by trauma or by the presence of irritants such as carbon particles or turpentine. Under the stimulus of these irritants papilla-like projections are formed by the ectoderm. With destruction of ectoderm, proliferation of mesodermal cells may form projections upon the surface, or with destruction of the mesothelium small masses of cells may project into the body cavity. Endothelium of a blood vessel may be stimulated to form masses of cells projecting into the lumen. Proliferation of cells below particulate matter that has entered the tissue may carry it to the external surface of the embryo or into the body cavity.

In early embryos, 3 days old, there is phagocytosis of particulate matter by mononuclear cells which have the characteristics of histiocytes and like them engulf and digest erythrocytes and other cells. In older embryos it is evident that these cells are in large part derived from perivascular cells with basophilic cytoplasm which have the structural characteristics of hemocytoblasts.

Granulocytes which are first formed in the somatopleure in contact with the yolk have little if any part in the reaction that follows the introduction of an inflammatory irritant into the tissues of early embryos and are first seen in small number at the site of inflammation in embryos from 6 to 8 days old.

Granulocytes that accumulate about an inflammatory irritant during embryonic life are in great part formed locally. The action of the irritant stimulates extravascular cells with the characteristics of hemocytoblasts to form acidophilic granules (granuloblasts). These cells, dividing by mitosis, produce at the site of inflammation myelocytes and mature polymorphonuclear granulocytes. At a very early period of development cells, of which the relation to the perichondrium suggests that they will take part in the formation of bone marrow, appear to be especially susceptible to transformation into granulocytes.

Cells morphologically resembling hemocytoblasts and widely distributed in the tissues of the embryo may be transformed by appropriate stimuli into histiocytes (macrophages).

Within a few days preceding hatching (17th to 19th day of embryonic development) inflammation assumes the character of postembryonic inflammation and granulocytes accumulate promptly and in large number by migration from blood vessels.

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INFLAMMATION IN EMBRYONIC LIFE

II. INFECTION OF CHICK EMBRYOS WITH AVIAN TUBERCLE BACILLI *

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The avian tubercle bacillus is well suited to the study of inflammation in chick embryos because it is pathogenic for fowls and produces in them well known changes. In the following experiments the reaction produced by the microorganism has been studied both in the chorioallantoic membrane and in the tissues of the body of the embryo.

When Goodpasture and Anderson¹ inoculated the chorioallantoic membrane of the chick with avian tubercle bacilli, embryos that were 6 days old when inoculated died within 4 days, but older embryos lived until the time of hatching. After 24 hours polymorphonuclear leukocytes and a few mononuclear cells were found at the site of inoculation and after 48 hours mononuclear cells had increased in number. Both kinds of cells ingested tubercle bacilli, and some mononuclear phagocytes ingested leukocytes containing tubercle bacilli. In mononuclear cells tubercle bacilli were found in great number, and the authors believed that the microorganism multiplied within them. Tubercle bacilli were found in mesodermal cells that were far distant from larger accumulations of tubercle bacilli. Following inoculation, Costil and Bloch² found tubercle bacilli within epithelial cells of the ectoderm. Human tubercle bacilli produced, 7 days after inoculation, collections of cells with little resemblance to tubercles. Lesions produced by B. C. G. after 7 to 9 days showed some evidence of retrogression.

In embryos, inoculated with human tubercle bacilli when 12 days old and observed 6 days later, Moore³ has described the formation of tubercles with caseation and giant cells. Atypical tubercles were produced by bovine tubercle bacilli. Avian tubercle bacilli invaded the mesoderm and were found in mononuclear phagocytes.

METHODS

Eggs, after different periods of incubation, prepared by the procedure described in the preceding article,⁴ have been inoculated with cultures of avian tubercle bacilli. Quantities of wet bacilli varying from 0.05 to 0.2 mg. have been used for inoculation. Sections stained with carbol fuchsin and counterstained with light green have been used to

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demonstrate tubercle bacilli. They have been compared with immediately adjacent sections cut in series and stained with hematoxylin and eosin-azure in order to demonstrate cellular structure in greater detail.

INFLAMMATION CAUSED BY AVIAN TUBERCLE BACILLI

Changes in the Membranes

When eggs, incubated for 6 days, were inoculated with avian tubercle bacilli and examined 6 (no. 159) or 8 (nos. 149 and 150) hours later, acid-fast bacilli were recognizable upon the surface of the chorioallantoic membrane in clumps usually surrounded by erythrocytes. A few were seen in ectodermal cells still attached to the membrane, others were in desquamated ectodermal cells and some were in round cells whose character was not definable. In embryos examined 8 hours after inoculation, tubercle bacilli were occasionally found in the mesoderm in or upon mesodermal cells. Twelve hours after infection (no. 151) some ectodermal cells, of which a few contained tubercle bacilli, had undergone proliferation and formed small projecting papillae. In the underlying mesoderm tubercle bacilli were seen in or upon mesodermal cells, and proliferation of these cells had occurred so that they were more numerous than elsewhere. On the surface of the ectoderm were round mononuclear cells containing tubercle bacilli, and these were in part, at least, histiocytes, for occasionally erythrocytes and tubercle bacilli were found within the same cell. Among the proliferating ectodermal and mesodermal cells of the chorion and among mononuclear cells found on the surface, granulocytes were moderately abundant, and several of them contained a few tubercle bacilli. Granulocytes on the surface had a bilobed or trilobed polymorphous nucleus, whereas in the underlying tissue granulocytes with a single vesicular nucleus were readily found and were most abundant about small blood vessels.

In an embryo 6 days old, 24 hours after infection (no. 152), ectoderm was lost in small areas and in others the cells had proliferated so that the margin about the defect was thickened. A few tubercle bacilli were found in the swollen ectodermal cells nearby. Below the site where the ectoderm was lost, mesodermal cells had multiplied in a circumscribed area, and here tubercle bacilli were found, but they were abundant only near the surface of the exposed mesoderm. Some were in cells that had anastomosing processes and were fibroblasts, but most of them formed compact clumps filling the cytoplasm of round cells of which the nucleus could be seen if tubercle bacilli were not too numerous. Mature granulocytes with lobed nuclei were found among the proliferating mesodermal cells and upon the surface of the mesoderm at

sites where ectoderm had been lost. An occasional granulocyte contained tubercle bacilli in small number.

In an embryo 6 days old, when examined 24 hours after infection (no. 169), tubercle bacilli had entered the chorioallantoic cavity and were found within the flat cells lining it. These cells had proliferated to form projecting mounds in which mesothelial and underlying mesoblastic cells were no longer distinguishable. The uppermost cells contained many tubercle bacilli, and cells, of which the cytoplasm was filled with bacilli, had become free in the overlying cavity. Tubercle bacilli that had penetrated downward into the mesoblast were found in contact with mesodermal cells and their anastomosing processes, and some bacilli were within the cytoplasm of these cells. Among the proliferating cells a few granulocytes were found. In a part of the membrane where it overlies the yolk, tubercle bacilli were abundant and here granulocytes were numerous. About blood vessels near the yolk, granulocytes were in process of formation. Mononuclear cells with basophilic cytoplasm contained a few round acidophilic granules (granuloblasts), and similar cells with numerous granules and no basophilic stain (myelocytes) were seen.

In an embryo 6 days old when infected, and killed 3 days later (no. 181), the only tubercle bacilli that were recognizable were within desquamated mesothelial cells free in the chorioallantoic cavity. Nevertheless, in places mesothelial and mesoblastic cells had undergone proliferation and were crowded together. Where this change had occurred, many mature granulocytes with lobed nuclei were found. They were seen within small blood vessels and were often adherent to the intima. In this part of the membrane there was no new formation of granulocytes, but in the membrane overlying the yolk sac hemocytoblasts filled the lumina of small blood vessels and similar cells were found outside of them. Acidophilic granules appeared in the basophilic cytoplasm of these cells and active new formation of granulocytes was evidently in progress.

In another embryo of the same age at the time of infection and examined 5 days later (no. 182), tubercle bacilli had multiplied actively and were found upon the surface of the ectoderm within desquamated cells, in ectodermal cells still attached and upon or within some of the underlying mesodermal cells. Where tubercle bacilli were abundant there was proliferation of both ectodermal and mesodermal cells. Granulocytes were very numerous and occasionally they contained tubercle bacilli. About small blood vessels granulocytes were in process of formation and cells with basophilic cytoplasm contained a few acidophilic granules which were round, larger and less brightly stained than

the elongated granules of mature avian granulocytes. Cells with the characters of myelocytes, as well as mature polymorphonuclear granulocytes, were found within the lumina of small vessels in the affected area.

In an embryo (no. 184), 11 days old at the time of injection and examined 48 hours later, there was active proliferation of ectodermal cells with formation of projecting mounds of cells and in places these cells had undergone keratinization. No tubercle bacilli were found except upon the surface, and here within granulocytes a few acid-fast bacilli were found. Granulocytes were abundant in the hyperplastic ectoderm and in the underlying mesoderm, and in the latter there was very active new formation of them. Granuloblasts were so numerous that 92 were counted about one small blood vessel cut tangentially, and here two of them were undergoing mitosis.

In one place within the mesoderm there was a lesion resembling an abscess, and here mature granulocytes with a few other cells occupied almost an entire field under low-power magnification. About this focus was a zone in which mononuclear cells predominated, although polymorphonuclear granulocytes were numerous. These mononuclear cells, in part with anastomosing processes, resembled embryonic fibroblasts and were closely crowded together. Less numerous were cells with basophilic cytoplasm resembling immature histiocytes. Granulocytes were in process of formation in the tissue surrounding the abscess, granuloblasts being found about small blood vessels and occasionally within their lumina. With the acid-fast stain, tubercle bacilli were not found within the area where granulocytes were most numerous nor in the surrounding zone of cell accumulation, but outside of the latter in apparently unaltered mesoblast a few tubercle bacilli were found in contact with mesodermal cells.

Changes in the Body of the Embryo

When avian tubercle bacilli were introduced into the body of an embryo (no. 168) 6 days old, they were found 6 hours later at the site of a defect in the ectoderm and in contact with the mesoderm. The only change that had occurred was some proliferation of ectodermal cells causing thickening of the ectoderm next to the defect. Tubercle bacilli that entered the mesoderm of embryos (nos. 163 and 164) 7 days old were found 6 hours after injection free in the mesoderm or in contact with mesodermal cells, but no reaction to their presence was evident.

In an embryo (no. 161) 6 days old when inoculated, and killed 24 hours after infection, tubercle bacilli were found in the mesoderm at a place where overlying ectoderm had been destroyed. Tubercle bacilli

were in contact with, and within, mesodermal cells with anastomosing processes. There had been some proliferation of these cells, as indicated by their increased number when compared with adjacent mesoderm and by the presence of mitotic figures. Here no macrophages and no granulocytes were found. In a circumscribed focus within the mesoderm close to the spinal cord, tubercle bacilli were present in such number that they were readily recognized with low-power magnification. Most of these tubercle bacilli were in round mononuclear cells and filled their cytoplasm. In the periphery of the focus tubercle bacilli were in contact with mesoblastic cells, but their number was small. Eosin-azure staining showed the presence of many round cells with basophilic cytoplasm, but granulocytes were not demonstrable.

In the same embryo tubercle bacilli had entered the blood stream, for within a small blood vessel adjacent to the focus just described a mononuclear cell contained several tubercle bacilli and in the liver were two foci in which cells had accumulated and in which tubercle bacilli were abundant. One of these foci was sharply defined because cells of mesoblastic type had replaced the columns of liver cells. Here round cells contained tubercle bacilli in great number, often filling the cytoplasm. Cells resembling fibroblasts contained the microorganism in smaller number, and fibers like those of reticulum were seen between the cells. In another place liver cell columns were intact and endothelial cells were apparently proliferating to form round cells that contained tubercle bacilli in large number. Restraint of the spread of tubercle bacilli was feeble; a few were found in flat endothelial cells lining small vessels, and some were present within liver cells.

In another embryo of the same age (no. 181), examined 48 hours after infection, there was evidence of dissemination by way of the blood. In this instance tubercle bacilli were found in an endothelial cell of the liver and several clumps of bacilli were within glomeruli of the kidney.

When tubercle bacilli had entered the body cavity of the embryo they were found in cells of the mesothelial lining. In one embryo (no. 152) 6 days old when infected and examined 24 hours later, mesothelial cells over the surface of the liver contained them, and within the cavity they were seen in mononuclear cells which were apparently in part desquamated lining cells. An occasional granulocyte was found in the abdominal cavity. In an embryo (no. 162) 48 hours after infection, necrosis of cells with nuclear fragmentation had occurred in the central part of a group of proliferating mesothelial cells in contact with the spleen. A few tubercle bacilli were found at the periphery of the necrotic area, but a larger number were in the immediately adjacent meso-

thelial cells, some of which were crowded with them. It is probable that some of the cells containing tubercle bacilli found within the body cavity were histiocytes, for in embryos (nos. 162 and 181) examined 48 hours after infection, mononuclear cells in this cavity contained erythrocytes as well as tubercle bacilli.

Embryos 11 days old at the time of infection and examined after 48 hours (no. 184) and after 81 hours (no. 185) have afforded opportunity to observe the local formation of granulocytes. In the former, tubercle bacilli were widely scattered in the mesoderm below a defect in the ectoderm of the leg. Here cells had accumulated in great number and in one small focus granulocytes were so numerous that the lesion resembled an abscess. Outside of this focus granulocytes were less numerous and mononuclear cells predominated. The latter were in part proliferating mesodermal cells and in part round mononuclear cells with basophilic cytoplasm. Tubercle bacilli were found in the abscess-like focus, in places filling the cytoplasm of mononuclear cells. At the periphery of the abscess where mononuclear cells predominated tubercle bacilli were not found, but in the relatively normal mesoblast outside of this area they were seen in or upon mesoblastic cells. In the zone in which mononuclear cells were abundant, new formation of granulocytes was proceeding actively, acidophilic granules being seen in mononuclear cells with basophilic cytoplasm (granuloblasts).

In embryos 18 days old at the time of inoculation and killed 24 hours later (nos. 214 and 216), bacilli had entered the subcutaneous tissue of the leg and the inflammatory reaction had the usual character of inflammation in birds and mammals. The tissue was edematous, and granulocytes had accumulated in considerable number. Round cells with a round or indented nucleus were present in smaller number, and some of them had ingested granulocytes. Both granulocytes and mononuclear cells had ingested tubercle bacilli, and occasionally it was evident that mononuclear cells contained granulocytes in which tubercle bacilli were recognizable. Granulocytes were seen within and about small vessels approximating capillaries in size and in places were engaged within the wall of the vessel. Migration of granulocytes was evidently in progress.

DISCUSSION

Tubercle bacilli introduced into the amniotic cavity of embryos 6 or 7 days old invade ectodermal cells and may multiply within them. Hyperplasia of ectoderm may produce projecting papillae. On the contrary, the microorganism may cause necrosis of the ectoderm, and where ectoderm is destroyed, or perhaps without destruction of it, tu-

bercle bacilli may penetrate the mesoderm. Here they may be found in contact with fibroblasts or their anastomosing processes or within the cytoplasm of these cells. Their relation to the cells is like that observed with carbon particles.⁴ At first there is no evident response to the presence of the organism, but within 12 hours mesoblastic cells proliferate. Bacilli are found within mesothelial cells lining the body cavity. The scant reaction that has occurred suggests that cells may have been invaded by the microorganism.

Within 12 hours after infection, tubercle bacilli are found in round cells with round or indented nuclei. The probability that these cells are histiocytes is increased by the observation that some of them contain both tubercle bacilli and erythrocytes or occasionally nuclear fragments.

In the chorioallantoic membrane of embryos 6 or 7 days old, granulocytes make their appearance within 12 hours after infection at sites where tubercle bacilli have caused proliferation or necrosis of ectoderm or of mesoderm, and a few of them may contain one or several tubercle bacilli. These cells have the polymorphous nuclei and elongated acidophilic granules of the mature granulocytes of fowls. In embryos of this age new formation of granulocytes is found during the early stages of the ensuing reaction only in that part of the membrane overlying the yolk where they are formed normally. In these early embryos that have lived 2 to 5 days after infection, granulocytes in process of formation have been found about small blood vessels at the site of injury. Evidently some of the granulocytes that accumulate here are formed locally. Granulocytes accumulate in the tissue adjacent to the injury; and granuloblasts, some in mitosis, are abundant, though none are found in corresponding parts of the membrane elsewhere. It is possible that a few granulocytes formed normally throughout the deeper part of the membrane reach the lesion by migration from blood vessels, but most of them are formed by cells that are stimulated by the irritant to form granuloblasts, and these in turn to form mature granulocytes. The cells from which these granuloblasts arise have a round nucleus and basophilic cytoplasm and resemble hemocytoblasts. Primitive cells, which form either erythrocytes, granuloblasts, or histiocytes, are indistinguishable under the condition of our study.

When avian tubercle bacilli have entered tissues of the body of embryos 6 or 7 days old they may cause proliferation of ectodermal or of mesodermal cells and in places necrosis of ectodermal cells may occur. After 24 hours a circumscribed lesion may be produced in the mesoderm. It is characterized by localized proliferation of embryonic fibroblasts and the appearance of many sharply defined round cells with round vesicular nuclei and basophilic cytoplasm. It is noteworthy that

most of the tubercle bacilli are contained in these round mononuclear wandering cells, whereas relatively few are found in or upon the fixed cells with anastomosing processes. Granulocytes accumulate less rapidly than in the membranes of the embryo and few, if any, are found after 24 hours in lesions produced by the microorganism. The lesion that is formed by proliferation of fibroblasts and accumulation of mononuclear phagocytes does not resemble a tubercle because it lacks the epithelioid cells that give the tubercle its characteristic form.

When tubercle bacilli have entered the body cavity they are found within the flat mesothelial cells that line it, and these cells undergo proliferation. It is probable that they are passively invaded, because tubercle bacilli have entered underlying liver cells as well. A focus of necrosis may be found within a mass of proliferating mesoblastic cells and is presumably produced by the action of the bacilli.

Tubercle bacilli in some instances have found their way into the blood stream and have been transported to the liver, where they are found in endothelial cells of blood vessels, and to the kidney, where they are lodged in glomeruli. In one experiment in an embryo 6 days old and examined 48 hours after inoculation, tubercle bacilli had been distributed by the blood stream, and lesions with some resemblance to tubercles were seen in the liver. In circumscribed foci, mononuclear cells contain tubercle bacilli in large number and are mingled with proliferating embryonic fibroblasts and newly formed collagen fibrils. Cells that are in contact with the endothelium of capillaries and are apparently analogous to Kupffer cells are proliferating and in part, at least, produce isolated mononuclear cells, of which the cytoplasm is filled with tubercle bacilli. The nodule that is formed has replaced the pre-existing columns of liver cells, but it differs from a tubercle because epithelioid cells are not found. No granulocytes have been seen in these lesions.

Accumulation of granulocytes in lesions of embryos 11 days old has been found 2 and 3 days after infection. In one instance granulocytes have assembled in such great number that the lesion resembles an abscess and in the periphery of this focus there has been active localized new formation of granulocytes. Granuloblasts in considerable number are dividing by mitosis.

In early embryos, preceding the tardy cellular reaction that ensues, there is scant resistance to the multiplication of avian tubercle bacilli and to invasion by them for they are found in ectodermal cells, in or upon fixed cells of the mesoderm, within mesothelial cells and even within liver cells. With the appearance of mononuclear phagocytes, which are found long before granulocytes appear, tubercle bacilli are

often seen in such great number in their cytoplasm that multiplication of the microorganism within the macrophage is probable. After some time, mature granulocytes appear in the lesion and are derived in great part at least from granuloblasts formed locally in response to the presence of the infectious agent. Under some conditions that are not definable, granulocytes are so abundant that the lesion has the appearance of an abscess. Granulocytes ingest tubercle bacilli, but only a few are found within them. In lesions where granulocytes are numerous, few tubercle bacilli are found, and it is probable that their presence is indicative of a reaction capable of retarding invasion by the avian tubercle bacillus.

In embryos 18 days old, that is, shortly before hatching, inflammation caused by the avian tubercle bacillus, like that following introduction of carbon particles or turpentine into the tissues, is characterized by accelerated accumulation of granulocytes by way of the blood vessels and resembles that of postembryonic life.

SUMMARY AND CONCLUSIONS

Avian tubercle bacilli, introduced into the membranes or into the tissues of early chick embryos, invade both ectodermal and mesodermal cells and cause accelerated proliferation of them. Under some conditions necrosis may ensue.

In early embryos up to 6 days of age there is at first tardy reaction to the presence of the microorganism, but mononuclear wandering cells containing tubercle bacilli make their appearance after approximately 12 hours. The great number of tubercle bacilli within them is probably the result of intracellular multiplication.

In early embryos a few granulocytes formed in the chorion in contact with the yolk sac may reach the site of inflammation by way of the blood stream, but most of those that accumulate about the microorganism in embryonic membranes or later in tissues of the body of the embryo are formed locally. Cells with the morphological character of hemocytoblasts are directly stimulated by the infectious agent to form granuloblasts characterized by basophilic cytoplasm containing a few acidophilic granules. These granuloblasts multiply by mitosis and in turn produce locally both myelocytes and mature granulocytes.

Circumscribed nodules are produced in the tissues of the embryo by proliferation of embryonic fibroblasts and accumulation of mononuclear wandering cells which ingest tubercle bacilli, but the lesion does not have the characteristics of a tubercle because epithelioid cells are not formed.

Granulocytes mobilize in increasing number during the last few days of embryonic life by migration from blood vessels and inflammation assumes its postembryonic character. Resistance to multiplication and invasion of avian tubercle bacilli increases with local increase in the number of granulocytes.

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ACQUIRED BICUSPID AORTIC VALVES WITH RETRACTED HORIZONTAL RAPHE *

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Although in most bicuspid aortic valves the raphe which divides the conjoined cusp is retracted into the sinus of Valsalva, the commissural attachment is not appreciably lowered. Nevertheless, certain examples occur in which the entire raphe is situated deep in the sinus of Valsalva and has a horizontal upper border attached to the aortic wall at a point distinctly below the normal position of the original commissure. This constitutes a distinct type of acquired bicuspid aortic valve.

REPORTS OF CASES

Case 1

A white male, 59 years old, was admitted to the hospital on March 4, 1940, and died on March 9, 1940. He complained of severe epigastric pain radiating to the back and shortness of breath of 5 days' duration. The heart was enlarged and the rhythm irregular but there were no murmurs. The blood pressure was 125 systolic and 60 diastolic. No history of rheumatic fever was obtained. The clinical diagnosis was either mesenteric thrombosis or dissecting aneurysm of the aorta.

The main pathologic diagnoses were idiopathic medial necrosis of the aorta with dissecting aneurysm of the thoracic and abdominal portions, left hemothorax (2.8 L.), cardiac hypertrophy and dilatation (700 gm.), syphilitic aortitis, and rheumatic heart disease with chronic endocarditis of the left atrium, mitral and aortic valves and formation of an acquired bicuspid aortic valve.

The bicuspid aortic valve consisted of one large cusp, 5 cm. in length, formed by complete fusion of the right and noncoronary cusps, and a smaller left cusp, 2 cm. long (Fig. 1). The triangular space between the two fused cusps on their ventricular aspect was virtually obliterated. The conjoined cusp was evenly subdivided at commissure B † by a narrow fibrous raphe measuring 5 by 2 by 1 to 2 mm.‡ This was markedly retracted and had practically a horizontal position at the

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† The following nomenclature of the aortic valve is used: The aortic cusps are designated according to the situation of the coronary arteries as the left, the right and the noncoronary cusps. The left-right commissure is referred to as commissure A, the right-noncoronary commissure as commissure B, and the left-noncoronary commissure as commissure C.

‡ These measurements indicate respectively the length (from origin to insertion), the width and the height (from the floor of the sinus of Valsalva to the superior surface) of the raphe.

bottom of the sinus of Valsalva. Proximally it originated from the aorta only 2 mm. above the attachment of the aortic valve and, after a linear course, it inserted into the base of the conjoined cusp. The outer surface was rounded, symmetric, approximately of uniform width and revealed no fissure. Just above the raphe there was a barely perceptible and irregular longitudinal elevation of the aorta.

Both aortic cusps were slightly thickened, especially the outer part of the conjoined cusp near the raphe. There was no calcific deposit. The free edge of the conjoined cusp presented a concave aspect toward the aorta. Commissures A and C showed no change. The right coronary ostium was situated 4 mm. above the commissural level while the left was in the usual position.

A longitudinal microscopic section through the middle of the raphe showed dense connective tissue.* Slight vascularity with capillaries and arterioles was present in the basal portion of the distal segment, just above the subaortic angle. The annulus fibrosus at the proximal extremity of the raphe was entirely anterior to the terminal elastic wedge of the aorta.†

The aortic cusps were the seat of diffuse fibrosis and also showed reduplication of the elastica in the ventricularis layer. However, no vascularity, exudate, or calcific change was present.

There was nodular thickening of the mitral valve along the line of closure. Microscopically the leaflets showed fibrosis and thick-walled blood vessels in the free portion. Although the left atrium was grossly negative, sections revealed elastic reduplications of the endocardium. The tricuspid and pulmonary valves showed no significant gross or microscopic change. The pericardium and myocardium were not remarkable.

Case 2

A white male, 64 years old, was admitted to the hospital on January 10, 1941, and died on January 15, 1941. He complained of severe substernal pain and shortness of breath of 1 week's duration. Examination of the heart was unsatisfactory because of marked pulmonary edema. The blood pressure was 100 systolic and 90 diastolic. No history of rheumatic fever was obtained. The clinical diagnoses were coronary thrombosis, myocardial infarction and acute pulmonary edema.

The main pathologic diagnoses were marked coronary arteriosclerosis with complete thrombotic occlusion of the left descending and right circumflex vessels, remote and recent myocardial infarction, cardiac

* Microscopic sections were studied after hematoxylin and eosin staining, the Weigert technic for elastic tissue and the combined Weigert and van Gieson methods for elastic and connective tissue.

† When the annulus is anterior to (or in front of) the aortic wedge, it is separated by the latter from the pericardium. If the annulus be posterior to (or behind) the wedge, it is in contact with the pericardium.

hypertrophy and dilatation (425 gm.), and chronic rheumatic heart disease with mitral, tricuspid, pulmonary and aortic valvulitis, formation of an acquired bicuspid aortic valve and calcific disease of the aortic valve with stenosis.

The aortic valve consisted of two cusps of equal size, each measuring 3.5 cm. in length (Fig. 2). One was a combined right and noncoronary cusp which presented an uninterrupted concave aspect toward the aorta. Commissure B was markedly retracted in the sinus of Valsalva and at its site was a centrally located, almost horizontally disposed raphe, calcified throughout its length. The raphe measured 8 by 2 to 3 by 3 mm., arose proximally from the aorta 3 mm. above the attachment of the valve and inserted distally into the base of the conjoined cusp. Its surface was nodular and irregular owing to calcific deposit and showed no fissure. There was no lesion of the aortic wall of the sinus of Valsalva above the raphe.

Both aortic cusps were rigid and showed calcific deposit. The sinuses of Valsalva were partly filled with calcified nodules. At commissure A there was slight fusion between the left and right cusps. Commissure C showed no change. The coronary ostia occupied their usual positions.

A transverse section of the raphe 3 mm. from its proximal end showed numerous calcific nodules but no vascularity or exudate. A longitudinal section of the entire raphe revealed diffuse calcific deposit. Vascularity and exudate were found principally in the ventricularis layer and especially distally in the region above the subaortic angle. Proximally the aortic media terminated entirely behind the fibrous tissue of the annulus. The calcific nodules in the aortic cusps were accompanied by focal vascularity with capillaries and exudation of lymphocytes.

The mitral, tricuspid and pulmonary valves, the left atrial endocardium, and the pericardium showed no significant gross change. Of numerous sections of the mitral valve, a few revealed vascularity and fibrosis of the ring and proximal free portion. The pulmonary valve was similar, while the tricuspid showed diffuse vascularity extending almost to the line of closure. There were no rheumatic lesions in the left atrium or myocardium.

Case 3

No clinical history was available.

The main pathologic diagnoses were marked coronary arteriosclerosis with complete thrombotic occlusion of the right circumflex artery, remote posterior basal infarct of the left ventricle, and chronic rheumatic heart disease with mitral and aortic valvulitis, formation of an acquired bicuspid aortic valve and calcific disease of the aortic valve.

The bicuspid aortic valve consisted of one cusp 4 cm. in length, formed by complete fusion of the right and noncoronary cusps and a smaller left cusp 3 cm. long (Fig. 3). The triangular space between the two fused cusps was obliterated. The conjoined cusp presented an uninterrupted concave aspect toward the aorta. It was evenly subdivided by a horizontal calcified ridge measuring 15 by 2 to 7 by 5 mm. This was situated deep in the sinus of Valsalva, its proximal end being attached to the aorta 8 mm. below the commissural level and its distal extremity inserting into the conjoined cusp midway between the ring and free edge. There was lateral bulging of the middle of the raphe resulting in a peculiar ovoid form. The outer surface was slightly nodular and revealed no fissure. The aorta above the proximal end showed no significant change.

Both aortic cusps were slightly thickened. There was fusion of the left and right cusps with calcific deposit in the raphe. Nodules of calcification were also present in the sinus of Valsalva. Commissure C showed no change. The coronary ostia occupied their usual positions.

Transverse sections of the raphe, 3 mm. from the proximal and the distal ends respectively, showed a similar picture. The calcific deposit was marked. There were vascularity and exudate in the lateral basal regions, *i.e.*, the attachments of the cusps, and in the distal raphe the vessels extended into the outer portion of the ventricularis layer.

A longitudinal section of the middle of the raphe revealed dense connective tissue containing numerous calcific nodules. Most of the calcium was deposited on the aortic side in the fibrosa layer. Vascularity was most prominent in the distal portion along the ventricularis layer, especially in and near the attachment of the valve. There were several thick-walled arteries. The aortic media was behind the annulus fibrosus at the commissural attachment.

The aortic cusps showed calcific nodules in all layers, accompanied by an infiltration of lymphocytes, and vascularity with capillaries and arterioles. There were prominent subaortic elastic reduplications.

The mitral valve was grossly negative except for a ridge of calcium extending from the noncoronary aortic cusp to the ring and base of the anterior leaflet. Microscopic sections showed focal calcific deposit, lymphocytic exudate and vascularity of the proximal free portion of both leaflets. The tricuspid and pulmonary valves, the left atrium, the pericardium and myocardium revealed no gross or microscopic evidence of rheumatic fever.

Case 4

A white man, 63 years old, was admitted to the hospital on March 28, 1941, and died on April 15, 1941. He gave a history of progressive shortness of breath for the

past 4 years and ankle edema for 1 year. Twenty years ago he was told by a physician that he had heart trouble. There was no history of rheumatic fever. The heart was enlarged, the rhythm regular and the blood pressure 110 systolic and 80 diastolic. Over the aortic area was a rough systolic murmur transmitted upward, and a faint aortic second sound but no diastolic murmur. The roentgenogram showed calcification in the region of the aortic valve. The clinical diagnoses were rheumatic heart disease with calcific aortic stenosis, auricular fibrillation and congestive heart failure.

The principal diagnoses at autopsy were chronic rheumatic heart disease with aortic valvulitis and formation of an acquired bicuspid aortic valve, calcific disease of the aortic valve with stenosis, and cardiac hypertrophy and dilatation (630 gm.).

The aortic valve consisted of two cusps of equal size, each measuring 3 cm. in length (Fig. 4). One was a combined right and noncoronary cusp, evenly subdivided by a raphe at commissure B. The raphe consisted of a calcified bar measuring 14 by 3 by 3 to 4 mm. and formed a horizontal ridge deep in the sinus of Valsalva. Its proximal attachment to the aorta was situated 1.2 cm. below the commissural level and after a linear course it inserted into the lower third of the conjoined cusp. The outer surface was slightly nodular and showed no fissure. There was no suggestion of a ridge in the aorta above the proximal end.

Both aortic cusps were rigid and practically immobile due to extensive calcific deposit. The sinuses of Valsalva were partly filled with calcific nodules. Commissures A and B showed no change. The coronary ostia were in the usual position.

Transverse microscopic section of the proximal raphe revealed calcific change but no vascularity or exudate. A transverse section of the distal raphe was similar except that the lateral basal regions contained capillaries and arterioles. Longitudinal microscopic section of the middle of the raphe revealed diffuse deposit of calcium in all layers, especially on the aortic side. Capillaries and an occasional arteriole were found along the base in the ventricularis layer. The terminal media of the aorta was distorted by the calcific process and calcified nodules even extended up behind it for a short distance. Nevertheless the elastic wedge remained posterior to the annulus fibrosus.

The aortic cusps revealed fibrosis, calcific deposit, diffuse vascularity and infiltration of lymphocytes. There were well developed elastic reduplications, some of multiple type, in the subaortic angle.

The mitral valve was grossly normal except for extension of a few calcific nodules from the aortic valve to the anterior leaflet. Microscopically there was no evidence of rheumatic fever. The tricuspid and pulmonary valves, the left atrium and the pericardium showed no significant gross or microscopic change.

Case 5

A white male, 64 years old, was admitted to the hospital on December 5, 1941, and died suddenly on December 12, 1941. He complained of progressive shortness of breath and edema of 1 year's duration. For the past 10 years he had had moderate dyspnea on exertion. There was no history of rheumatic fever. The heart was enlarged, the rate 40, the rhythm regular and the blood pressure 132 systolic and 98 diastolic. Over the aortic area was a harsh systolic murmur transmitted to the neck and absent aortic second sound, but no thrill or diastolic murmur. An electrocardiogram showed complete heart block. The clinical diagnoses were arteriosclerotic heart disease, aortic stenosis, cardiac enlargement, heart block and cardiac failure.

The main pathologic diagnoses were rheumatic heart disease with chronic mitral, pulmonary and aortic valvulitis with formation of an acquired bicuspid aortic valve, calcific stenosis of the aortic valve with extension of calcification into the membranous interventricular septum, and cardiac hypertrophy and dilatation (525 gm.).

The aortic valve consisted of two cusps of equal size, each measuring 3.8 cm. in length (Fig. 5). One was a conjoined cusp formed by fusion of the left and noncoronary cusps and it presented a continuous concave aspect toward the aorta. Deep in the sinus of Valsalva was a calcific horizontal ridge situated 1.2 cm. from commissure A and 2.6 cm. from commissure B. It measured 10 by 3 to 5 by 4 mm., arose proximally from the aorta just above the attachment of the aortic valve and inserted distally into the base of the conjoined cusp. There was no lesion of the aorta above the proximal end. The distal portion of the raphe was wider than the proximal. The outer surface was nodular because of calcific deposit and showed no fissure.

Both aortic cusps revealed diffuse calcific disease and were rigid and practically immobile. Calcific nodules filled the sinuses of Valsalva and also projected from the cusps at the line of closure. There was fusion of the left and right cusps for a distance of 5 mm. Commissure B showed no change. The left coronary ostium was situated 5 mm. above the commissural level while the right ostium was in its usual position.

Longitudinal microscopic section of the raphe of the conjoined cusp showed calcific deposit throughout its length. There was vascularity in the vicinity of the calcium and also along the base of the raphe in the ventricularis layer. Several thick-walled arteries were present. There was a prominent focus of vascularity distally near the subaortic angle. The aortic cusps showed diffuse fibrosis, calcification, foci of cartilage and osteoid tissue, exudate of lymphocytes and vascularity with capillaries and arterioles. There were occasional thick-walled arteries.

The mitral valve showed thickening at the line of closure and focal calcific deposit in the posterior leaflet. Microscopic sections revealed

chronic mitral and pulmonary valvulitis. There were no other stigmas of rheumatic fever.

SUMMARY OF CASES

The patients were all white males and their ages ranged from 59 to 64 years. In 2 cases there was a clinical diagnosis of aortic stenosis. None of the patients gave a history of rheumatic fever.

Gross Appearance of the Cusps

In 4 cases the conjoined cusp was formed by fusion of the right and noncoronary cusps and in 1 by fusion of the left and noncoronary cusps. In 3 cases the conjoined cusp was equal in length to the remaining cusp and in 2 it was larger. The conjoined cusp was evenly subdivided by the commissural raphe in 4 instances; in 1 case it was subdivided unequally into a small noncoronary and a larger left coronary segment. In all instances there was marked or almost complete obliteration of the triangular space below the raphe, and the concave aspect of the free edge of the conjoined cusp toward the aorta was continuous. The other commissures of the aortic valve showed fusion in 3 cases, *i.e.*, at commissure A in each instance.

The aortic cusps showed pathologic change in all cases. In 3 instances there was marked calcific disease and aortic stenosis. Calcific change without stenosis was present in 1 case and thickening without calcific deposit in 1 case.

The Commissural Raphe

Grossly the raphe consisted of a firm ridge of tissue situated deep in the sinus of Valsalva and occupying a horizontal or nearly horizontal position in the sinus (perpendicular to the long axis of the aorta). The raphe occurred at commissure B in 4 cases and at commissure C in 1 case. The proximal attachment to the aorta was generally only a few millimeters above the attachment of the aortic valve and the distal insertion was into the base or midportion of the conjoined cusp.

In 4 of the 5 cases the raphe was calcified throughout its length. It was usually either of uniform width or wider distally than proximally; in 3 cases the shape was irregular owing to calcific deposit and the surface was nodular. None of the lesions showed a fissure in the surface. The aorta above the raphe was smooth in 4 cases, while in 1 case it showed a barely visible, longitudinal elevation. The latter was readily distinguished from the congenital ridge described below.

Microscopically the raphe revealed dense connective tissue and usually calcific nodules. There was no significant elastica. In the longi-

tudinal sections vascularity and sometimes exudate were present along the base in the ventricularis layer, especially distally, and were generally prominent in the region of the attachment of the valve. Transverse sections revealed vessels in the lateral basal region and especially in the distal segment. In all cases the aortic media terminated behind the annulus fibrosus at the proximal end of the raphe. In 2 instances there was irregularity of the wedge owing to calcific deposit and inflammation.

Lesions of Rheumatic Fever

Rheumatic stigmas were present in all aortic valves. Particular attention was paid to stigmas of the mitral valve and multiple microscopic sections of both the anterior and posterior leaflets were made. Grossly the valve was normal in 3 cases and showed thickening without commissural fusion in 2 cases. Microscopically, however, 2 of the 3 grossly normal valves showed inflammation in the form of vascularity of the free portion, fibrosis and exudate. In the series, 4 of the 5 cases revealed conclusive microscopic evidence of chronic mitral valvulitis, probably rheumatic. In 3 cases rheumatic lesions were observed in the heart elsewhere than in the mitral and aortic valves; *i.e.*, in the left atrium in 1 case, in the tricuspid and pulmonary valves in 1 case and in the pulmonary valve alone in 1 case.

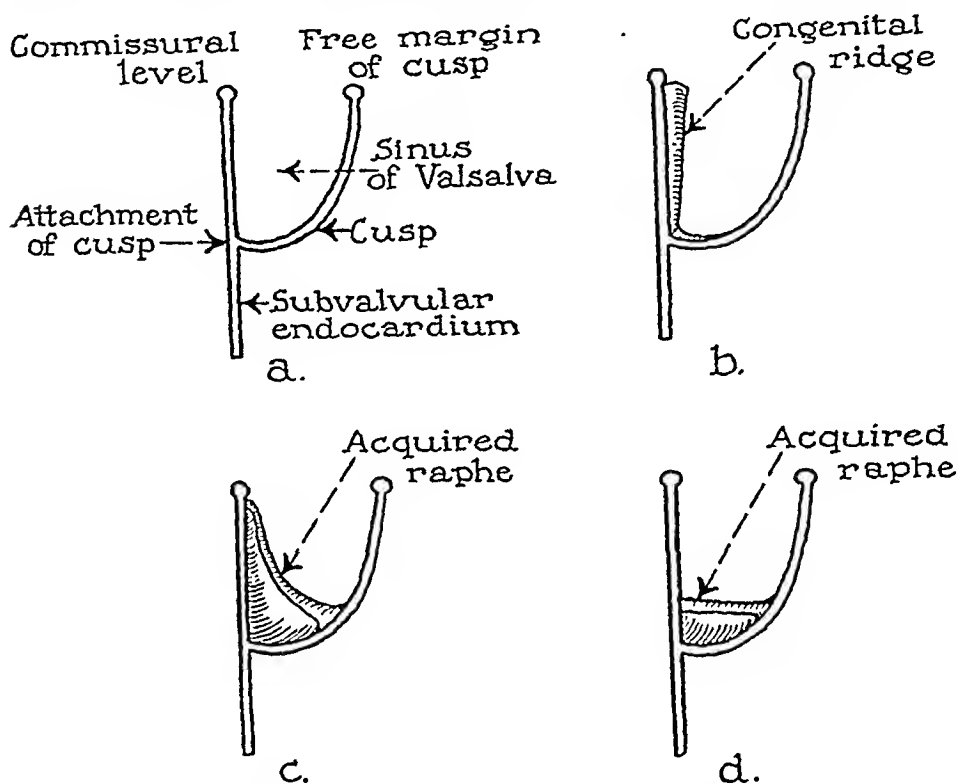
COMMENT

The bicuspid aortic valve described in this paper is produced by inflammatory fusion of two aortic cusps. Following adhesion certain changes occur: (1) the triangular space between the cusps is gradually obliterated; (2) the free margin of the conjoined cusp develops an uninterrupted concave aspect toward the aorta; (3) the commissural raphe is retracted into the sinus of Valsalva. Grossly the conjoined cusp then appears to be a single cusp.

The present lesion differs from the common variety of acquired bicuspid valve only in respect to the peculiar location and direction of the commissural raphe (Text-Fig. 1, d). This is uniformly retracted along its entire length so that it occupies a horizontal or nearly horizontal position at the bottom of the sinus of Valsalva. When retraction is especially marked, the raphe forms merely a small and inconspicuous ridge overlying the attachment of the aortic valve. Proximally it is attached to the aorta deep in the sinus and after a linear course inserts distally into the base of the conjoined cusp. The outer surface is smooth and rounded, or nodular and distorted owing to calcific deposit. Although not present in these cases, raphes of triangular shape suggesting

the outline of the fused cusps, and those showing a fissure with or without preservation of the cusp margins, probably occur.

The horizontal position of the raphe is evidently due to downward displacement of the proximal extremity from its original location at the commissural level. That the proximal origin of the raphe is actually situated at a commissure is indicated by the fact that the relationship between aortic media and annulus fibrosus is the same as occurs at a

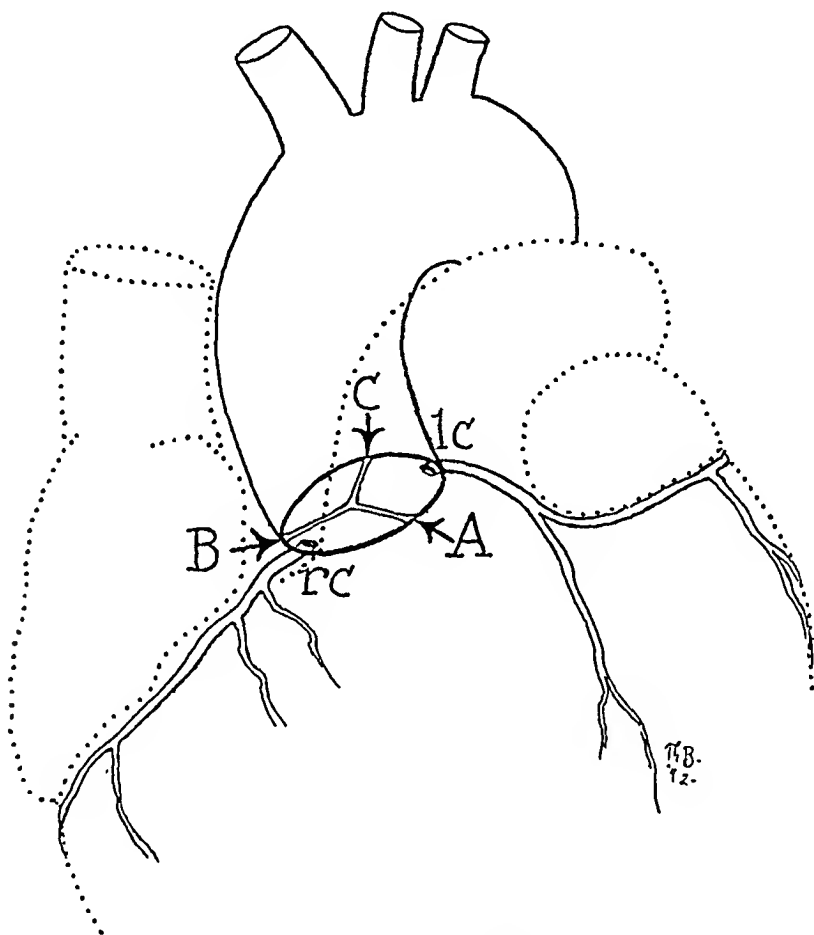


Text-Figure 1. Drawings of longitudinal sections of aortic valve to show the position in the sinus of Valsalva of the congenital ridge and the commissural raphe of acquired bicuspid aortic valves: a = normal valve; b = congenital ridge; c = acquired commissural raphe, usual type; d = retracted, horizontal type of acquired raphe.

commissure. As far as is known, location of a commissure at the bottom of the sinus of Valsalva does not occur normally.

The aorta, at about the point of attachment of the raphe, rather than the raphe itself, appears to be displaced downward. This displacement is presumably due to stretching of the aorta in this region, but the exact mechanism of the change of position is not clear. The fact that the lesion occurs most often at commissure B may be significant. In chronic rheumatic aortic valvulitis without bicuspid deformity, slight depression of commissure B is more frequent than at commissures A and C. There are somewhat hypothetic explanations, both physiologic and anatomic. The location of commissure B, to the right and in the con-

cave aspect of the longitudinal curve of the aorta and at a lower horizontal level than commissures A and C, is such that pressure exerted during diastole is possibly greater than at the other commissures (Text-Fig. 2); this effect might well be increased by the presence of disease in this region. It is also possible that the structure at commissure B is such that support against diastolic pressure is less than at commissure A or C.



Text-Figure 2. Drawing of aortic valve to show the position of the commissures *in situ*. The valve is inclined upward and to the left. Commissure B is situated to the right and in the concave aspect of the longitudinal curve of the ascending aorta, and at a lower horizontal level than commissures A and C.

In the usual type of acquired bicuspid aortic valve, the raphe is situated most often at commissure A, infrequently at commissure B and rarely at commissure C. The proximal extremity remains attached to the aorta at the commissural level, while the distal portion is retracted and inserts into the base of the conjoined cusp. Hence the direction in the sinus of Valsalva is generally oblique (Text-Fig. 1, c). Usually this raphe is symmetric, of greater width distally than proximally, and pre-

sents a rounded or smooth outer surface. However, the appearance may vary considerably, especially when there is superimposed calcific deposit, so that the raphe is irregular, nodular, or even distorted in shape.

Bicuspid lesions with low horizontal raphe are apparently much less frequent than those with oblique raphe, occurring in the ratio of about 1 to 5. A few additional cases have been encountered which appear to demonstrate an intermediate stage between those with no downward displacement of the commissural attachment and those described in this paper. In these intermediate cases the point of commissural attachment is slightly but not markedly below the upper border of the sinus of Valsalva.

Microscopically the commissural raphes of acquired bicuspid aortic valves are similar in appearance regardless of type.¹ They are composed of dense hyalinized connective tissue, may or may not show calcific deposit and have little or no elastica. In longitudinal sections vascularity and sometimes exudate are present in the ventricularis layer along the base of the raphe, especially in the distal segment, and are usually most prominent in the region of the attachment of the valve and the subaortic angle. In transverse section, especially of the distal portion, vessels may be seen in the lateral or basal region, corresponding to the attachment of the fused cusps.

Of importance is the relation of the annulus fibrosus to the terminal aortic wedge behind the proximal raphe. Although the raphes generally occur at the center of a conjoined cusp, they represent commissural lesions and hence should preserve the usual relation at the commissure, *i.e.*, termination of the aortic medial wedge posterior to the annulus. This was found to be true in all of the present cases, even though in two instances the terminal elastica was irregular and distorted by calcific deposit and inflammation. With very marked distortion, however, it may not be possible to determine this relation with certainty.

To be distinguished from acquired bicuspid aortic valves are those of congenital origin. The latter are uncommon in adults, and may be subdivided into those consisting of two normal cusps and those in which one of the cusps is divided by a ridge of congenital origin into two segments. The congenital ridge consists of a long, narrow, barlike elevation of the aorta, which projects only slightly into the sinus of Valsalva, is directed in the long axis of the aorta and is of uniform width and depth (Text-Fig. 1, b). Microscopically, it consists almost entirely of elastica whorled centrally and continuous laterally with that of the aortic media.²⁻⁴

A distinction between congenital and acquired bicuspid aortic valves

is not possible on the basis of the conjoined cusps themselves. In general appearance, size and location these may be entirely similar in the two lesions. The distinction depends on the differences in structure, both grossly and microscopically, between the congenital ridge and the acquired commissural raphe. These have already been indicated. Also of aid in differentiation is the aortic media-annulus fibrosus relation. In the acquired raphe the media lies behind the annulus, thus preserving the normal commissural relation. In the congenital ridge the annulus is generally overlapped both in front and behind by the terminal aortic wedge, with the posterior overlap lower than the anterior. Occasionally the annulus lies either entirely in front of the wedge or entirely behind it.

When calcific disease is present, the acquired lesion may be difficult to distinguish from the simple congenital bicuspid valve. This is especially true when the raphe is inconspicuous, situated very deeply in the sinus of Valsalva and largely obscured by calcific nodules in the sinus; the lesion might then appear to be of simple congenital type with calcific change. In most instances, however, the raphe can be identified grossly and established microscopically as an acquired commissural lesion by the aortic media-annulus fibrosus relation. If the raphe be completely obscured by the calcific deposit, a distinction between the two lesions may be impossible.

Occasionally, confluent nodules of calcification in the sinus of Valsalva form a ridge which simulates the commissural raphe. However, this pseudoraphe is grossly irregular and poorly defined in contrast to the sharply outlined true raphe. Moreover, should the ridge be situated in the central portion of the cusp, which is the usual position of the commissural raphe, microscopic section would reveal a terminal aortic media in front of the annulus rather than behind it. If the location is lateral to or near the commissure, microscopic study might not be of differential aid, since here the aortic media may lie behind the annulus normally.

The acquired bicuspid aortic valve is the result of an inflammatory process which is in all probability rheumatic in origin. Gross⁵ has reviewed the evidence for a rheumatic etiology. This rests on the structure of the commissural raphe and also on the presence of stigmas of rheumatic fever, both in the aortic valve and elsewhere in the heart.

Horizontal raphes, similar grossly and microscopically to the present lesion, occur in rheumatic aortic valves which show commissural fusion but not bicuspid deformity. Here, also, the horizontal position of the raphe is probably due to lowering of its commissural attachment. Such

valves resemble the bicuspid lesion, but differ from it in two respects: (1) the triangular space below the raphe is only partly obliterated, and (2) the concave aspect of the fused cusps is interrupted opposite the raphe where it is convex.

The pathologic changes in the bicuspid aortic valves, namely, diffuse thickening and calcific deposit, are morphologically indistinguishable from those which occur in rheumatic fever. This includes such items as fibrosis involving all layers of the cusps, location of the calcific nodules principally in the fibrosa layer, vascularity and exudate in the ventricularis layer, and the presence of thick-walled blood vessels with muscular coats in the attachment and free portion of the cusps. Most recent studies support the view that calcific disease of the aortic valve has an inflammatory basis and is not degenerative in origin.⁶⁻⁸

In hearts with chronic or healed rheumatic fever, the stigmas of the disease are usually widespread. There are characteristic gross and microscopic changes. The gross lesions include various degrees of thickening, shortening and commissural fusion of the valves, especially the mitral and tricuspid; thickening and adhesion of the chordae tendineae; nodular thickening and wrinkling of the left atrial endocardium above the posterior mitral leaflet, and fibrous pericardial adhesions, especially in the atrioventricular sulci. Characteristic microscopic lesions consist of vascularity, exudate and fibrosis in the ring and free portion of the valves, involving especially the auricularis layer of the mitral and tricuspid valves and the ventricularis layer of the semilunar valves; vascularity and reduplication of the elastica of the endocardium of the left atrium and the subaortic angle, and Aschoff nodules in the myocardium.

In the present study, only unequivocal lesions were accepted as rheumatic stigmas. Attention was directed particularly to inflammation of the valves, especially the mitral and tricuspid. In situations other than the aortic valve, gross manifestations of rheumatic fever were present in only 2 cases and consisted of chronic or healed nondeforming mitral valvulitis. Microscopically, there were conclusive rheumatic lesions outside the aortic valve in 4 cases. The fifth case presumably represents an instance of rheumatic heart disease with residual lesions demonstrable only in the aortic valve.

The clinical significance of the condition is that of acquired bicuspid aortic valves in general. The bicuspid lesion usually occurs in hearts which are the seat of only mild or slight rheumatic disease.¹ In most cases, for example, the accompanying disease of the mitral valve is of the nondeforming type rather than productive of mitral stenosis. Furthermore, the bicuspid aortic valve is only rarely encountered in chil-

dren or young adults dead of florid rheumatic fever or in adults who have chronic rheumatic heart disease with marked deformity of two or more valves and cardiac failure.

The bicuspid valve results from fusion of two adjacent aortic cusps to form a single conjoined cusp. This pathologic change probably takes place within a relatively short period of time during childhood or early adult life. It is probable that functional alteration occurs, *i.e.*, stenosis due to adhesion, or insufficiency due to retraction of cusps, but is transient in nature and disappears when the bicuspid lesion is complete. The single and fused cusps evidently undergo stretching and as a result become functionally competent in systole and diastole. Thus the bicuspid lesion *per se* has no permanent effect on the heart.

Individuals with acquired bicuspid aortic valves are, however, prone to develop calcific disease of the valve. This development is an integral part of the underlying rheumatic valvulitis. The calcific deposit leads to rigidity of the cusps and in severe cases to stenosis of the aortic valve and eventual cardiac decompensation. Since the deposition of lime salts occurs slowly over a considerable period of time, functional valve disease is generally not apparent until later life. This was true of the cases in the present study.

Another disease, which may be superimposed on the acquired bicuspid aortic valve, is bacterial endocarditis, either acute or subacute. Presumably this is due largely to the rheumatic origin of the bicuspid lesion. Although none of the acquired lesions in this study showed bacterial disease, the latter is not infrequent in my experience in the usual form of acquired bicuspid valve. However, in general, bacterial endocarditis occurs less commonly in these valves than calcific disease.

SUMMARY AND CONCLUSIONS

Five cases of acquired bicuspid aortic valve are described. In each case the conjoined cusp presented a markedly retracted, horizontal raphe deep in the sinus of Valsalva. This low and unusual position of the raphe is evidently due to downward displacement of its commissural attachment.

The lesions are inflammatory in origin and in all probability due to rheumatic fever. The evidence for a rheumatic origin rests on the morphology of the commissural raphe and also on the presence of stigmas of rheumatic fever, both in the aortic valve and elsewhere in the heart.

Calcific disease of the aortic valve was present in 4 of the 5 cases and 3 of these showed aortic stenosis.

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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 42

- FIG. 1. Case 1, a white male, 59 years old, with an acquired bicuspid aortic valve. The raphe at commissure B is markedly retracted and lies at the bottom of the sinus of Valsalva, just above the attachment of the aortic valve.
- In this and succeeding figures, A, B and C represent the left-right, the right-noncoronary, and left-noncoronary commissures respectively, while lc and rc indicate the ostia of the left and right coronary arteries respectively.
- FIG. 2. Case 2, a white male, 64 years old, with an acquired bicuspid aortic valve, the seat of calcific disease with stenosis. The raphe at commissure B is retracted, horizontal and calcified.
- FIG. 3. Case 3, an acquired bicuspid aortic valve, the seat of calcific disease. The raphe at commissure B consists of a calcified ridge of peculiar shape situated deep in the sinus of Valsalva.
- FIG. 4. Case 4, a white male, 63 years old, with calcific stenosis of an acquired bicuspid aortic valve. At commissure B is a low, prominent, horizontal raphe which is calcified.
- FIG. 6. Case 5, a white male, 64 years old, with calcific stenosis of an acquired bicuspid aortic valve. At commissure C is a calcified horizontal raphe, situated just above the attachment of the aortic valve.



Koletsky

Bicuspid Aortic Valves

BACTERIAL ENDOCARDITIS DUE TO CLOSTRIDIUM WELCHII *

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While bacterial endocarditis is most frequently caused by streptococcal, staphylococcal, or pneumococcal infections, other organisms in great variety have been reported as etiological agents in small numbers of cases. Shiling,¹ in 1939, reviewed the cases of bacterial endocarditis in which he felt there was sufficient clinical, pathological and bacteriological evidence to establish the causative organism. He was able to compile a list of twenty-six different bacteria as the offending organisms in bacterial endocarditis and added two additional organisms which had occurred in cases of his own. Neither in Shiling's review of the literature nor in a search of the literature since that time was there encountered a single case in which endocarditis was caused by *Clostridium welchii* (*perfringens*). It is true that Janbon and associates^{2, 3} reported three cases of *Cl. welchii* septicaemia, associated with "endomyocarditis" in two cases and endocarditis in another, but the diagnosis of cardiac involvement was made on the finding of positive blood cultures, changing heart murmurs and myocardial failure alone. In none of these cases was an autopsy performed to confirm the clinical diagnosis. The purpose of the present communication is to report a case in which the diagnosis of acute bacterial endocarditis due to *Cl. welchii* was confirmed by post-mortem examination.

REPORT OF CASE

V. H., a married woman, 34 years old, was admitted to the gynaecological service of the Montreal Maternity Hospital on January 3, 1941, complaining of menorrhagia. She had a previous history of Sydenham's chorea at 11 years of age, with subsequent heart damage which had not been incapacitating at any time. She had a normal menstrual history until August, 1940, when she began to have a change in the character of her menstrual periods. There were no other significant findings in her personal or family history.

On admission, the temperature was 99° F.; pulse, 88; respiration, 20; blood pressure, 182/106. There were harsh apical presystolic and mid-diastolic murmurs and similar murmurs over the left sternal border. The posterior lip of the cervix was thick and hard. A specimen of the cervix was reported as carcinoma solidum.

On January 6th radium was inserted into the cervix and this was removed on January 7th. One hour after the removal of the radium the temperature was 101° F. The patient continued thereafter to have a daily fever varying from 102° to 104° F. associated with chills. Sulphanilamide was administered commencing on the second day of the fever. A swab of the cervix taken on January 14th, 1 week after removal of the radium, was reported positive for *Cl. welchii*. Blood taken on

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January 16th yielded a pure culture of *Cl. welchii*. At this time jaundice was noticed; the skin was hot and flushed, and tender petechiae were present on the right palm and fingers. There was a harsh basal systolic and a high-pitched prolonged diastolic murmur and it was thought that the patient had septicaemia and endocarditis. Repeated plasma and whole blood transfusions were given. Administration of sulphathiazole was substituted for the sulphanilamide therapy on January 17th. On January 25th the spleen and liver became palpable for the first time and the spleen was tender on pressure. At this time 20,000 units of anti-gas-gangrene serum were given followed by another 5,000 units on January 29th. On February 1st, signs and symptoms of occlusion of a large artery to the left lower extremity appeared. Gangrene of the left leg developed which necessitated amputation on February 19th. Abscesses were found in the necrotic anterior tibial muscles from which *Cl. welchii* was grown in pure culture. The patient became sensitized following the injections of anti-gas-gangrene serum and no further antiserum could be given, as attempts to desensitize the patient failed. *Cl. welchii* was grown in pure culture on two additional occasions from blood taken on February 21st and March 5th. Numerous transfusions were given but the patient became steadily weaker and died on March 10th, 9 weeks after the first symptoms of infection.

Gross Examination

A complete post-mortem examination (no. 10843), excepting the contents of the cranial cavity, was performed 7 hours after death. In the following summary all of the pertinent findings are included.

The heart, weighing 525 gm., showed hypertrophy and dilatation of both right and left sides. The free margins of the mitral valve leaflets were firm and thick and the chordae tendineae of the mitral valve were shortened, thickened and inserted into the mitral valve by broad attachments. There was a rough patch of thickened endocardium on the posterior wall of the left auricle. The aortic valve cusps were stiff and opaque with markedly thickened margins. Between the noncoronary and the left coronary cusps of the aortic valve and extending over their adjacent ventricular surfaces was a brown, friable, mulberrylike vegetation over an area of 2 by 3 cm. There was ulceration and perforation of the noncoronary cusp of the aortic valve (Fig. 1).

The lungs were heavy and hyperemic. The bronchi contained thick bloody mucus and the mucosa was congested. An embolus partly filled the lumen of the superior mesenteric artery. A segment of the terminal portion of the jejunum, about 1 foot in length, was hyperemic, oedematous and covered by a thin fibrinous exudate. The lower pole of the spleen, the adjacent stomach, colon and omentum, joined by adhesions, formed the walls of an abscess cavity containing thick greenish yellow pus. The spleen was enlarged, weighing 290 gm. The capsular surface presented many yellow, irregular, bulging areas measuring up to 2 cm. in diameter, some of which were firm, corresponding to underlying areas of infarction. Some, however, were fluctuant due to abscess formation. The right kidney presented two firm yellow areas of infarction

in the cortex, 1.5 cm. in diameter, and an abscess of similar size. In the cervix there were two deep lateral fissures. The mucous surfaces of the cervical canal and uterus were opaque, white and finely granular, covered with a film of purulent-appearing fluid. Almost the whole length of the left common iliac artery was filled with a thrombus which at one point was soft and purulent. Similar material was present in the right hypogastric artery. There was a recent mid thigh amputation of the left lower extremity. The dorsum of the right foot was somewhat blue and desiccated in appearance. In none of the tissues examined was there any grossly detectable gas formation.

Microscopical Examination

The heart was studied histologically in a number of sections taken to include myocardium, endocardium and the mitral and aortic valve cusps. There was a hyaline connective tissue thickening of the aortic valve cusp with many well formed arterioles in its thickened base. On the aortic surface of the valve and towards the free margin of the cusp there was a layer of young fibrous connective tissue covered by endothelium. On the ventricular surface of the valve cusp, extending from the free margin to the base, was a thick mass of fibrin and platelets containing many degenerating neutrophils and a few minute areas of coarsely granular calcium deposit. Beneath this vegetation, especially at the base of the valve cusp and in the adjacent myocardium, there was a heavy infiltration of polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, associated with marked vascular dilatation. The deeper layers of the vegetation showed some organization. In a section of the aortic valve stained by Glynn's⁴ method for the demonstration of Gram-positive and Gram-negative organisms, great numbers of large Gram-positive bacilli possessing the morphology of *Cl. welchii* were found throughout the vegetation and were especially abundant in its deeper parts (Fig. 2). A careful search with the oil immersion lens failed to reveal organisms of any other morphology. The mitral valve presented marked hyaline fibrous connective tissue thickening. In the base of the valve there were many thick-walled muscular arteries. The endocardium of the left auricle was thickened and sparsely infiltrated with small round cells. There were numerous areas of perivascular fibrosis in the heart muscle, in some of which definite Aschoff cells could be distinguished.

Both lungs showed chronic passive congestion with more recent hyperemia and hemorrhage into the alveoli. There was an extensive fresh central necrosis of the liver lobules. A section of the jejunum showed early coagulative necrosis. In sections of the spleen and kidney multiple

septic infarcts were found. Some of the intralobular arteries related to the renal infarcts were occluded by emboli. The infarcts in both organs were bordered by zones of hemorrhage with many pyknotic and fragmented nuclei, among which considerable numbers of degenerating polymorphonuclear leukocytes could be distinguished. The necrotic tissue in the infarcted areas showed marked disorganization of the normal architecture and was irregularly infiltrated with polymorphonuclear leukocytes in various stages of degeneration. One infarct in the spleen and one in the kidney showed complete disintegration and liquefaction of the central part which contained tissue debris and many clearly recognizable, degenerating polymorphonuclear leukocytes. Sections of the cervix showed a marked fibrosis with complete loss of epithelium. No evidence of carcinoma could be seen. In sections of the left common iliac artery, the lumen was filled with thrombotic material showing early organization of the periphery. The arterial walls showed changes ranging from a moderate mononuclear inflammatory reaction to complete destruction. Several sections of the tibial arteries taken at the time of amputation of the left leg contained an organized thrombus while others showed more recent thrombi. In addition to the sections of the aortic vegetations stained for bacteria, sections of the spleen, kidney, liver and thrombus in the left common iliac artery were also stained by Glynn's⁴ method. Except for one vessel in the kidney which contained Gram-positive rods similar to those of the aortic vegetations, no bacteria were seen possessing the morphology of *Cl. welchii*, and in none of these sections were bacteria of any other morphology found on careful search.

In summary, therefore, there was found an acute bacterial endocarditis of the aortic valves which showed evidence of previous rheumatic damage. The septic infarcts of spleen and kidneys, as well as the gangrene of the jejunum and lower extremities, were interpreted as the result of septic embolism from the aortic vegetations.

Bacteriological Studies

Cultures of the heart's blood taken post-mortem yielded a pure growth of *Cl. welchii*. Pus from the perisplenic abscess gave a heavy growth of *Cl. welchii* with a few Gram-positive micrococci which were regarded as contaminants.

The second and third cultures of the blood taken during life and the culture of the heart's blood taken at autopsy were grown in media containing p-amino-benzoic acid. The organisms obtained during life from the blood and from the abscess in the amputated leg, as well as those grown post-mortem and referred to as *Cl. welchii*, were nonmotile or-

ganisms possessing the morphological and cultural characters of *Cl. welchii*. Their biochemical reactions listed below served to identify them definitely as *Cl. welchii*.

Liquefaction of gelatin...+	Glucose...acid and gas formation
Meatgas formation	Maltose...acid and gas formation
Litmus milkacid, clot, gas, stormy fermentation	Lactose...acid and gas formation
Löffler's serumno digestion	Sucrose...acid and gas formation
H ₂ S+	Mannite.. negative
	Salicin....negative

DISCUSSION

Failure to find any previous autopsy reports of bacterial endocarditis due to *Cl. welchii* indicates that this is probably a rare condition. Therefore, the question whether *Cl. welchii* was really the etiological agent must be given the most careful study and all other possibilities excluded. Bacteriological studies, both clinical and post-mortem, and the pathological findings all support the conclusion that *Cl. welchii* was the etiological agent. The first blood culture taken 9 days after the onset of fever, two other blood cultures taken during life and the post-mortem blood culture yielded *Cl. welchii* and no other bacterial growth either aerobically or anaerobically. The abscesses of the amputated leg and the perisplenic abscess also yielded *Cl. welchii*. A few Gram-positive micrococci recovered from the perisplenic abscess were regarded as contaminants. Prolonged search with the oil immersion lens of sections of the aortic vegetations and other infected tissues stained for bacteria revealed only large Gram-positive rods possessing the morphology of *Cl. welchii*. Because of the gross resemblance of the aortic vegetations to those of bacterial endocarditis caused by *Streptococcus viridans*, the possibility was considered that the endocarditis had been initiated by *Str. viridans* or some other bacterial infection with subsequent implantation of *Cl. welchii* as a terminal invader. While this possibility cannot be excluded with complete certainty, there is not one whit of evidence in favour of it. There was no clinical history of an illness which could be interpreted as being due to septicaemia previous to the insertion of radium into the cervix, and a blood culture taken 9 days after the onset of clinical septicaemia yielded a pure culture of *Cl. welchii*, a result which was repeated on two further occasions during life and also post-mortem. No other pathogenic organisms were recovered in any of the bacterial cultures during life or after death. The objection might be raised that the growth of other pathogenic organisms in cultures had been inhibited by the presence of sulphanilamide or sulphathiazole in the circulating blood. This objection can be met by

pointing out that two of the three blood cultures taken during life and the one blood culture taken at autopsy were grown in media to which p-amino-benzoic acid had been added. Moreover, it is scarcely conceivable that sulphonamide therapy could completely eradicate bacterial pathogens from a large endocardial vegetation when once the organisms had become established there. Nevertheless, thorough search of histological sections of the vegetation on the aortic valve appropriately stained to demonstrate bacteria failed to reveal the presence of any bacteria other than those possessing the morphology and staining properties of *Cl. welchii*. Thus, the conclusion that *Cl. welchii* was the sole bacterial etiological agent seems inescapable.

The fact that *Cl. welchii* was growing in a vegetation bathed in oxygenated blood is not inconsistent with the known biological characters of this organism. Walbum and Reymann⁵ have pointed out that a total absence of oxygen is not necessary for the growth of *Cl. welchii* and there are many recorded examples of the survival and growth of this organism in the blood stream in cases of *Cl. welchii* septicaemia. In such cases the infecting organisms represent strains of relatively low virulence and the patient may survive for a considerable time or even recover.^{2, 3, 6-8} It has been known for some time that in cultures of *Cl. welchii* there may appear variants which remain constant for years, each strain possessing specific antigenic qualities. According to McGaughey,⁹ Orr, Josephson, Baker and Reed¹⁰ and Borthwick,¹¹ the different variants remain constant in their cultural, morphological and staining characteristics and toxin production. McGaughey stated that such variations may be responsible for the differences seen in cases of natural infection. The suppurative character of the reaction elicited by the *Cl. welchii* in the present case, resulting in abscess formation in the left lower extremity, right kidney, spleen and perisplenic region, is very unusual and is probably referable to a peculiarity of the particular strain of organism concerned in this instance. It is also peculiar that, although the organism produced gas in various culture media, none could be detected in any of the infected tissues.

The origin of the infection in this case was undoubtedly the genital tract and it seems probable that manipulation of the cervix during the insertion of radium played a part in initiating bacteremia. Russell and Roach¹² stated that in about 5.5 per cent of patients *Cl. welchii* can be grown from vaginal swabs, while Sadusk and Manahan¹³ placed this figure at 8.7 per cent. Cosgrove and Barry⁷ pointed out that the organisms in most of the cases must be of low virulence or more patients would die of gas bacillus infection after pelvic operations.

In view of the knowledge that rheumatic lesions of the heart valves

predispose to bacterial endocarditis due to other organisms, it appears highly probable that the presence of valvular lesions of rheumatic origin in the present case constituted an important predisposing factor in the development of endocarditis caused by *Cl. welchii*.

SUMMARY AND CONCLUSIONS

A case has been presented in which *Cl. welchii* septicaemia occurred after the insertion of radium into the cervix for treatment of carcinoma. At autopsy, an acute bacterial endocarditis of the aortic valve was found. Careful investigation led to the conclusion that *Cl. welchii* was the bacterial etiological agent. The autopsy findings also confirmed the clinical opinion that the endocarditis occurred in a heart previously damaged by rheumatic infection. In a careful search of the literature there was found no previous report of bacterial endocarditis due to *Cl. welchii* in which the diagnosis had been established by post-mortem examination.

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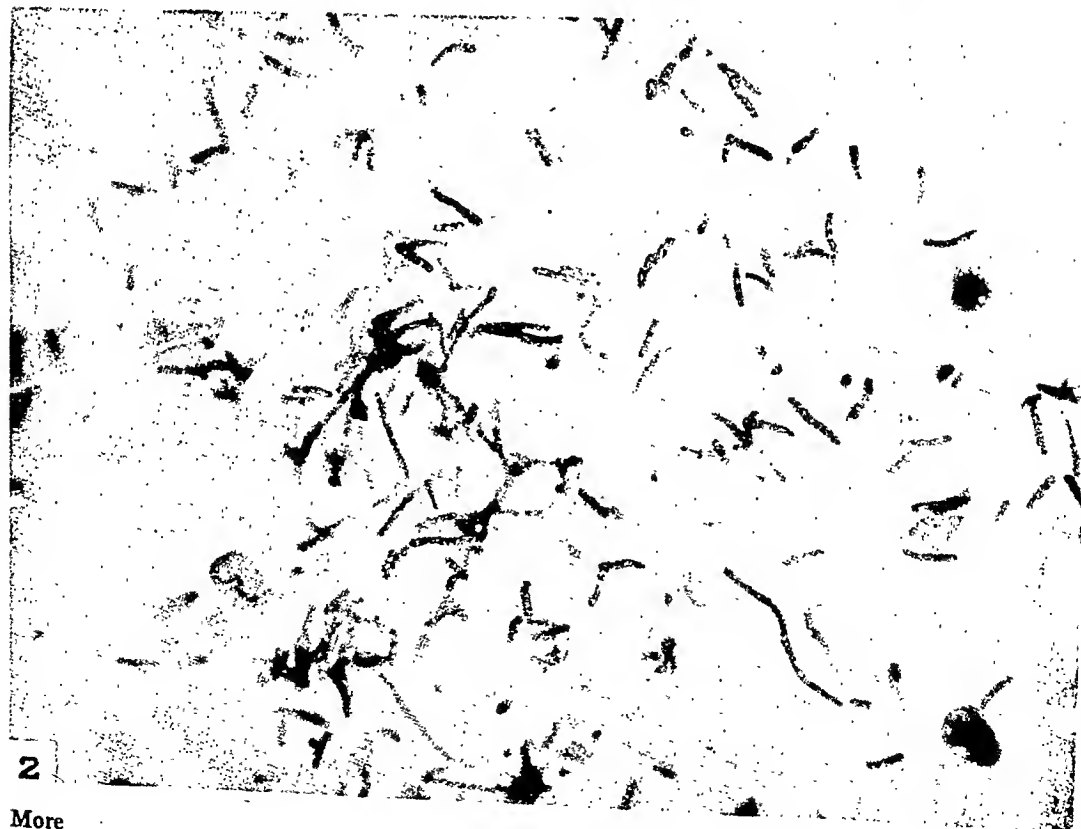
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DESCRIPTION OF PLATE

PLATE 43

- FIG. 1. Photograph of aortic vegetations. The left coronary cusp has been removed for section. The friable character of the vegetations may be noted as well as erosion of the noncoronary cusp and thickening of the uninvolved cusp.
- FIG. 2. Photomicrograph of a section of the aortic vegetations stained by Glynn's method to demonstrate bacteria. The bacteria in this field lay deep in the vegetation, were Gram-positive and possessed the morphology of *Clostridium welchii*. $\times 1500$.



More

Endocarditis Due to *Clostridium welchii*



HISTOCHEMICAL STUDIES ON TISSUE ENZYMES

III. A STUDY OF THE DISTRIBUTION OF ACID PHOSPHATASES WITH SPECIAL REFERENCE TO THE NERVOUS SYSTEM *

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INTRODUCTION

Acid phosphatases with an optimum *in vitro* pH of about 5 have been found in liver and spleen,¹ in the prostate,²⁻⁴ seminal fluid^{2, 5} and urine⁶ of adults, and in serum.^{7, 8} Nothing is known of the function of these enzymes in liver, spleen, or kidney. However, Gutman and Gutman⁵ reported that little or no acid phosphatase is present in the prostates of human beings and monkeys before puberty and that a marked increase occurs with puberty. They were able to produce an increase in the acid phosphatase content of the prostate by injection of testosterone propionate⁹ and have suggested that acid phosphatase may be important in the glycolytic phases of the reproductive process. These authors also found that the acid phosphatase of the serum of normal persons is not of prostatic origin,⁸ but that the marked increase in the acid phosphatase of serum which occurs in persons with metastasizing carcinoma of the prostate is due to prostatic phosphatase and is of value in the diagnosis of metastatic carcinoma of the prostate.¹⁰ Huggins and Hodges¹¹ have shown that orchiectomy caused a marked drop in the elevated acid phosphatase of patients with metastasizing prostatic carcinoma.

Gomori¹² has introduced a histochemical method for the demonstration of acid phosphatases in tissue and has shown that the enzyme activity is localized in the glandular epithelium of the prostate. He has also described the distribution of acid phosphatases in other tissues, notably liver, spleen, adrenal and kidney, and has recorded the differences in distribution between acid and alkaline phosphatase.

The present study concerns the distribution of acid phosphatases in normal and neoplastic tissues of the nervous system, for which no histochemical data have hitherto been reported.¹² In the course of the work certain changes and precautions were found to be necessary to insure maximal enzyme activity. Under these conditions, acid phosphatase was observed to be more widely distributed than had previously been found¹² and a detailed description of the distribution of the enzyme in several species is given.

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METHOD

The procedure for the demonstration of acid phosphatases, as outlined by Gomori,¹² is based on the deposition of lead phosphate at the site of the enzyme activity, when a tissue section is incubated at 37° C. with an organic phosphate ester in the presence of lead ions buffered at pH 4.7.

Tissues were fixed in cold, concentrated, absolute acetone for 24 hours, using three changes of acetone. The tissues were next placed in absolute alcohol for 24 hours, followed by 24 hours in toluol, and subsequently embedded in paraffin.

Sections were cut at 10 μ and mounted on slides. These were run through two changes of xylol followed by two changes of absolute alcohol, washed rapidly in tap water and transferred to the incubating mixture warmed to 37° C.

The incubating mixture was freshly prepared for each experiment and consisted of:

- 12 cc. acetate buffer at pH 4.7
- 10 cc. lead nitrate, M./10
- 74 cc. distilled water
- 4 cc. 3.2% sodium- β -glycerophosphate

Sections were incubated for 19 to 24 hours. Sections adjacent to those stained for acid phosphatase were placed in a similar mixture of substrate which contained 0.001 to 0.01 M. sodium fluoride, which served as a control by inhibiting the enzyme activity. Upon removal, all sections were washed with 6 to 8 changes of distilled water during $\frac{1}{2}$ hour to remove any excess lead. The sections were then transferred to a dilute solution of ammonium sulfide for 2 minutes, washed thoroughly in tap water, rinsed with distilled water, counterstained lightly with Harris's hematoxylin and with eosin and mounted in balsam.

In the finished section, the staining varies from light brown to black depending on the relative amounts of the enzyme present in the tissue. Control sections incubated for as long as 48 hours in the presence of as low as M./1000 fluoride gave uniformly negative results.

Yeast nucleic acid used at pH 4.7 was also found to be a suitable substrate¹³ but the staining was less intense than with sodium- β -glycerophosphate under the same conditions. Glucose-1-phosphate was also used successfully.

At pH 4.7, the enzyme was unaffected by M./100 phenobarbital, M./500 sodium azide, M./500 iodo-acetic acid. M./100 magnesium ions had a slight inhibiting effect as did M./100 phlorhizin.¹⁴

Contrary to Gomori's observations,¹² sodium- β -glycerophosphate was found to be a suitable substrate provided too high a concentration was avoided. A comparative study using adjacent sections and the same concentration of substrate showed that much less staining was obtained in unit time with a mixture containing 52% α -glycerophosphate as substrate than with pure β -glycerophosphate. Optimal staining was obtained at pH 4.7 to 5.1.

Unless conditions are adequately controlled, the poor penetrating power of the acetone used as a fixative may lead to erratic results. To determine the effective penetrating power of acetone, sections from several blocks of tissue were taken at increasing distances from the surface of the block. It was found that the most uniform results were obtained with sections at depths of 150 to 500 μ below the surface of the tissue in the paraffin block. At greater depths staining was much less uniform. The first few sections of tissue also showed little reaction for enzyme and as a routine procedure to obtain optimum results, the first 100 μ of tissue after levelling off the paraffin block were discarded.

The optimal time of incubation was found to be 20 to 24 hours and with tissues which were not too carefully treated, 48 hours of incubation was sometimes neces-

sary. Gomori¹² incubated most of his sections for 6 hours and only in a few instances as long as 15 hours.

RESULTS

With these precautions, our results in general confirm the positive findings of Gomori.¹² However, because of the establishment of optimal conditions with respect to substrate, time of incubation and fixation of tissue, it has been possible to demonstrate acid phosphatase in tissues which Gomori reported as negative.

The organs of a group of freshly killed, apparently normal animals and normal and abnormal tissues removed at four routine human autopsies were studied for the presence and distribution of acid phosphatase. The animals included three rabbits, a guinea-pig and six mice. The human cases were three males, 10½, 39 and 50 years of age and a female of 75 years. The necropsies were performed from 3½ to 13 hours after death. The boy of 10½ years had an astrocytoma of the brain stem, the man 39 years old hypertension and arteriosclerosis with coronary occlusion, the man of 50 years a carcinoma of the lung which involved only the thoracic organs, and the woman a carcinoma of the colon and central lobular necrosis in the liver. A number of specimens of each organ were examined in most instances. The findings are recorded by organs or systems for the whole group. Any variations occurring in the different animals or due to the presence of a pathological condition are appended to each description.

In addition, a small group of primary and secondary tumors of the central nervous system was investigated for acid phosphatase content.

The use of the term "staining" or its synonyms in the following description refers to the demonstration of the presence of enzyme activity as carried out in the above procedure and the intensity of the stain may be taken as a rough indication of the degree of activity.

Central Nervous System. Throughout the brain and spinal cord, the neural tissue was moderately and diffusely stained. On this background, individual nerve cells stood out sharply (Figs. 1, 2 and 3), the nucleus and cytoplasm being stained most intensely. The number of stained nerve cells varied considerably from area to area. In general, it may be said that the larger neural elements were much more often and more deeply impregnated than the smaller ones. Staining of these larger elements occurred less often in the cerebral cortex, basal ganglia, thalamus and cerebellum than in the midbrain, pons, medulla and spinal cord. In the former group, the majority of nerve cells showed a more intense staining of the nuclei than of the cytoplasm, which stained moderately and diffusely. Single cells or groups of cells in the cerebral cortex, especially in the pyramidal layer (Fig. 3), were picked out sharply

through the intense staining of their cytoplasm, nucleus and parts of their dendrites and axon. In the corpus striatum, the large nerve cells were frequently intensely stained while the smaller ones stained like the majority of the cerebral cortical cells. In the thalamus, most of the cells stained moderately. In the cerebellum, the Purkinje cells and cells of the tectal nuclei were often picked out by their deep staining; the nuclei of the granule cells were intensely impregnated. In the mid-brain, pons (Fig. 17), medulla and spinal cord, the nerve cells forming the various nuclei were sharply impregnated so that the various cell groups stood out boldly.

The dendrites and axons of the very deeply impregnated neural elements could at times be followed for some distance (Figs. 3 and 7). Within the larger nerve cells, particularly in the motor elements, the neurofibrils stood out sharply, and could be followed in the cell processes as well. In the white matter, the axons were sharply outlined and deeply stained (Fig. 6). The myelin sheaths were unstained. The nuclei of all three types of glia—astrocytes, microglia, and oligodendroglia—stained well but their cytoplasm was uncolored. At times, it seemed that the processes of the astrocytes stained. The nuclei of ependymal cells were deeply, and their cytoplasm more lightly stained, while in the choroidal cells both were deeply impregnated. The walls of parenchymal blood vessels were unstained except for their nuclei. This was true, as well, for the leptomeninges with the exception of the larger leptomeningeal vessels which reacted as did other blood vessels.

Peripheral Nervous System. In the cranial nerves (Figs. 4 and 5), nerve roots, peripheral nerves (as seen in muscle), a sympathetic ganglion (human) (Fig. 9) and nerves encountered in various organs, the axons stained deeply and sharply. They stood out boldly even when the surrounding tissue was unstained. The nuclei of Schwann and endoneurial cells, but not their cytoplasm, were frequently stained and the myelin sheaths remained unstained.

Heart. The muscle nuclei stained deeply and the fibers moderately or lightly (Figs. 11 and 12). In one human case in which there were areas of myocardial fibrosis, these were clearly outlined due to the lack of staining of all but the nuclei of the connective tissue cells. The pericardium and endocardium were unstained except for the light staining of the nuclei of their cells.

Arteries and Veins. The nuclei of all of the mural elements and the bodies of the smooth muscle cells were stained. The media, therefore, was clearly outlined.

Capillaries. Only the nuclei stained.

Lung. The nuclei of the alveolar lining cells were stained. Occasionally the cytoplasm of single cells in the alveolar walls was moderately stained and in rare instances the cytoplasm of all the cells stained lightly. The nuclei and cytoplasm of the bronchial epithelium stained deeply as did those of the mucous glands on occasion (Figs. 18 and 19). The smooth muscle cells took the stain less intensely, the nuclei being impregnated more deeply than the cytoplasm. The nuclei of all of the other connective tissue elements stained but their cytoplasm remained unstained. The pleura was unstained.

Trachea. The lining cells of the mucosa showed moderate staining of their nuclei and light staining of their cytoplasm. In the serous and mucous glands only the nuclei stained. The smooth muscle stained as elsewhere (see blood vessels).

Spleen. The malpighian corpuscles stained more lightly than the remainder of the parenchyma, only the nuclei of their constituent elements being stained moderately. The only exception was the spleen of the woman of 75 years, with carcinoma of the colon and a reaction following blood transfusion, in which the corpuscles stained more deeply than the pulp. In the pulp, the nuclei of the lymphocytes stained deeply, while those of the cells of the reticulum, large mononuclear elements and multinucleated cells stained moderately. Sometimes the bodies of the last stained distinctly and the bodies of the large mononuclear elements lightly. The cytoplasm of the other cells was unstained. The capsule was unstained while the nuclei of the cells in the septa were stained.

Liver. The nuclei and cytoplasm of the hepatic cells were impregnated, the former more deeply (Fig. 14). The cytoplasm showed distinct coarse granulation. The nuclei of the Kupffer cells stained moderately, while their cytoplasm stained inconstantly and faintly; in some mice, however, the cytoplasm of the Kupffer cells stained well. Both the nuclei and cytoplasm of the bile duct epithelium were stained, the nuclei more deeply. In the large bile ducts there was a concentration of cytoplasmic staining near the free border of the cell. The nuclei of the connective tissue cells in the portal spaces were impregnated but the capsule was unstained.

In the woman of 75 years, the degenerating hepatic cells in the areas of central necrosis showed no staining of their cytoplasm and only irregular faint staining of their nuclei.

Pancreas. The islands of Langerhans in general stained somewhat more deeply than the acini (Fig. 16). The nuclei of the islet cells stained deeply and their cytoplasm moderately. The nuclei and cytoplasm of the acinar cells stained much less intensely but at times ap-

proached the depth of impregnation of the islet cells. The duct lining cells stained as did the acinar cells, or more lightly.

Stomach. Irregular staining of the cytoplasm of the epithelial cells and those lining the glands of the mucosa occurred. Contiguous cells often differed in their reaction, but this could not be correlated with cell types. The nuclei of these cells stained fairly regularly. In the woman of 75 years these mucosal cells all stained regularly and deeply. In the stroma, only the nuclei stained, while the muscularis showed deep staining of the nuclei and moderate staining of the bodies of its cells.

Esophagus. The stratified epithelium was chiefly unstained. Occasionally there was staining of the cytoplasm of the cells of the upper layers while their nuclei remained unstained.

Small Intestine. The cells lining the surface and the glands of the mucosa showed deep staining of their nuclei and moderate staining of their cytoplasm. In the stroma, only the nuclei stained, while the muscularis stained as did smooth muscle elsewhere.

Large Intestine. The mucosal epithelium and the cells lining its glands showed moderate staining of their nuclei and lighter staining of their cytoplasm. The mucosal stroma and the muscularis stained as elsewhere in the gastrointestinal tract.

Adrenal. The nuclei of both cortical and medullary cells stained deeply while the cytoplasm of the medullary elements was much more deeply stained than that of the cortical cells. At times, the glomerular zone of the cortex was more deeply impregnated than the rest. Cortical cells containing large amounts of lipid often appeared more lightly stained than the others due to the dispersion in their cytoplasm of positively staining, coarsely granular material.

Kidney. In the glomeruli as a rule only the nuclei stained. In some mice the epithelial cells of the glomerular loops and Bowman's capsule showed some staining of their cytoplasm. In the convoluted tubules there was moderate staining of both nuclei and cytoplasm. The descending arm of Henle's loop often showed intense staining of both nuclei and cytoplasm, while the loop and ascending arm stained similarly to the convoluted tubules or more lightly. In the collecting tubules the nuclei were deeply stained. The cytoplasm, chiefly at the cell margins, was intensely stained and this was greatest toward the lumen. In some mice the epithelial lining cells from the convoluted tubules to the ducts stained equally. In the woman of 75 years with a transfusion nephrosis there was no definite difference noted in the convoluted tubules. The ducts of Bellini exhibited deep staining of the nuclei of their cells while the cytoplasm stained lightly.

Bladder. The nuclei of the epithelial cells of the mucosa stained

moderately and the cytoplasm was unstained. Smooth muscle stained as elsewhere. The nuclei of the serosal cells stained lightly.

Prostate. The nuclei and cytoplasm of the cells lining the tubules stained very deeply (Fig. 13). The nuclei of the cells in the interstitial tissue stained moderately to deeply, but their cytoplasm was unstained except for moderate staining of the smooth muscle cells.

Testis. The cytoplasm and nuclei of both sustentacular and spermatogenic cells were stained. Of the latter the spermatogonia stained most deeply and the spermatids least. In most instances spermatozoa were unstained. In some of the mice the heads of the sperm stained deeply and occasionally the tails stained lightly. The nuclei of the interstitial cells were moderately stained while their cytoplasm remained unstained.

Epididymis. The cytoplasm and nuclei of the tubular epithelium stained moderately to deeply, the free borders of the cells being impregnated somewhat more intensely.

Seminal Vesicle. The epithelial lining showed deep staining of its nuclei and light staining of its cytoplasm.

Vas Deferens. Throughout the wall only nuclei stained, except for the smooth muscle which stained as elsewhere.

Uterus. The surface epithelial cells and cells lining the glands of the mucosa showed deep staining of their cytoplasm and nuclei (Fig. 15). The most intense cytoplasmic staining was at the free border of the cell. In the mucosal stroma only the nuclei stained. The smooth muscle stained as it did elsewhere.

Ovary. Only the nuclei stained in the capsule. The nuclei and cytoplasm of the ova stained moderately. The cytoplasm of the follicular cells stained similarly while their nuclei stained very deeply. In the ovarian stroma, the nuclei and cytoplasm stained moderately or the cytoplasm remained unstained.

Fallopian Tube. In the lining cells of the mucosa the nuclei stained very deeply and the cytoplasm moderately.

Thyroid. The nuclei of the acinar cells stained moderately and their cytoplasm lightly. The colloid material stained moderately or at times deeply.

Pituitary. All three types of cells in the anterior lobe stained, the nuclei more deeply than the cytoplasm. The intermediate zone, posterior lobe and capsule were unstained.

Parathyroid. The cytoplasm of all of the cells was stained rather lightly while the nuclei stained moderately.

Striated Muscle. The sarcolemma and muscle nuclei stained moderately. The muscle fibers stained lightly or moderately and their striations were clearly visible. At times the muscle fibers were unstained.

PRIMARY OR SECONDARY NEOPLASMS OF THE CENTRAL NERVOUS
SYSTEM AND ITS MEMBRANES

Twenty-three tumors were examined. Of these 11 were gliomas, 10 meningiomas, 1 a metastatic melanoma and the last a metastatic sarcoma. These were all specimens taken for biopsy and fixed in acetone directly after their removal.

Gliomas

Astrocytoma. Three tumors were examined. The nuclei of the neoplastic astrocytes stained deeply and their cytoplasm and often their processes stained moderately. In many areas only the nuclei stained.

Glioblastoma. Three tumors of this type were examined. The nuclei of all the varieties of cells in this neoplasm stained deeply while their cytoplasm and occasionally their processes stained moderately. The necrotic areas were unstained except for nuclear fragments which stained deeply.

Medulloblastoma. One tumor was examined. The nuclei of the tumor cells stained very deeply but their cytoplasm remained unstained.

Oligodendroglioma. Three tumors were examined. One did not stain. In the other two the nuclei stained deeply while the cytoplasm was unstained or stained irregularly (Fig. 8).

Papilloma of Choroid. The nuclei of the choroidal epithelium stained deeply and the cytoplasm moderately. Only nuclei were stained in the connective tissue and blood vessel walls.

Meningioma

Ten tumors were examined. Three were negative. In the other seven the nuclei of the tumor cells stained moderately to deeply (Fig. 10). In three of the seven the cytoplasm of these cells stained moderately and remained unstained in the others.

Other Tumors

Metastatic Melanoma. The nuclei of the tumor cells stained deeply while their cytoplasm remained unstained.

Metastatic Sarcoma (Source Unknown). The nuclei and cytoplasm of the neoplastic elements stained quite heavily.

In two of the human autopsies referred to in the preceding sections tumors were encountered. One was an oat-cell carcinoma of the lung and the other a carcinoma of the colon.

Carcinoma of Lung (Oat-Cell Type). The nuclei of the tumor cells stained deeply while their cytoplasm remained unstained.

Carcinoma of Colon. The nuclei of the neoplastic elements stained deeply and their cytoplasm moderately.

DISCUSSION

Evidence that the histochemical method for localizing acid phosphatases in tissues is specific for these enzymes has been obtained in a manner similar to that used with the histochemical method for alkaline phosphatase.¹⁵⁻¹⁸ Thus when the procedure is carried out on adjacent sections of tissue without the addition of sodium- β -glycerophosphate, only tissues containing calcium are stained; when carried out in the presence of M./1000 to M./100 fluoride, a known inhibitor of acid phosphatases, complete inhibition of enzyme activity is obtained. Histochemical determination of the optimum pH for enzyme action is in agreement with the optimum pH as determined chemically. Magnesium ions have an inhibitory effect on the action of the enzyme when tested chemically¹ or histochemically. Phlorhizin was found by Beck¹⁴ to have only a slight inhibitory effect and a slight effect was also obtained histochemically. In addition, the enzyme has been shown to be inactivated by alcohol and alcohol-fixed tissues have shown no indication of any enzyme activity.

In general it may be said that the activity of acid phosphatase is regularly demonstrable by the present method in the nuclei of cells of all tissues. This suggests that the enzyme may play a rôle in nuclear metabolism. In addition, it is encountered in the cytoplasm and processes of the cells of many organs. The nervous system is one of the most consistent sites of enzyme activity and here it is noted primarily in the nerve cell and its processes. With increasing size of the ganglion cells, their bodies show increasing acid phosphatase activity. The smallest nerve cells—the granule cells of the cerebellum and other areas—show little or no evidence of enzyme activity in their cytoplasm while the largest elements—the motor ganglion cells—exhibit the most. Axons contain considerable amounts of the enzyme and it is often demonstrable in the individual neurofibrils, while myelin sheaths are free of it. This raises the question of the possible relationship of acid phosphatase to the transmission of nervous impulses and this point is to be investigated further. While acid phosphatase activity was confined to the nuclei of glial cells, with the possible exception of astrocytic processes, the bodies of ependymal cells and more particularly of choroidal cells frequently contained the enzyme. The cytoplasm of the Schwann cells like that of the glial elements was free of it. The fact that acid phosphatase is regularly present in the cytoplasm of choroidal cells and less richly in that of ependymal cells may possibly indicate some common function, distinct from that of the glial cells in which the enzyme is absent.

In contrast, alkaline phosphatase¹⁹ is irregularly distributed in the

nervous system and not consistently present. It is diffuse in its distribution, absent in the nerve cells and its processes, regularly present in astrocytic fibers and constantly present in the vascular endothelium of this as of other organs. Whereas acid phosphatase is as rich in the human nervous system as in that of some of the lower animals, alkaline phosphatase is comparatively sparse except in blood vessel walls.

Because of the sharp and deep impregnation of axons and, to a lesser degree, of the individual neurofibrils in the central and peripheral nervous system, the method has some value as a stain for those structures. It has the advantage of rapidity over the other methods of staining axons and neurofibrils in blocks of tissue since it can be carried out in a few days as compared with several weeks required by the other methods. The stains for axons carried out on individual sections prepared from frozen or embedded tissues are usually most distinct in tissues fixed for a number of weeks; in this instance the acid phosphatase method also has the advantage of speed. The deep impregnation of the nerve cell body and its dendrites at times resembles that seen in Golgi stains and although it is often similarly irregular it is never as complete. The axons in peripheral nerves are regularly and brilliantly demonstrated.

Gomori¹² has described the occurrence of acid phosphatase activity in a variety of organs. By the use of the present method it becomes evident that such enzyme activity is present in additional sites than those recorded. It is present not only in the splenic pulp but in the malpighian corpuscles as well, although to a lesser degree. In the liver not only are the hepatic cells positive but the lining of the biliary ducts as well. The pancreas is constantly positive, the islet cells often containing more enzyme than the acinar elements. In addition to the general nuclear acid phosphatase in the lungs, the bronchial epithelium regularly contains the enzyme. The myocardium and striated muscle are irregularly positive while smooth muscle is almost always positive. The mucosal lining throughout the gastrointestinal tract was irregularly positive for the enzyme. As Gomori¹² has pointed out, the medulla of the adrenal is rich in acid phosphatase, but the enzyme is also constantly present in the cortex although in lesser amounts. The kidney is much more often positive than has been stated. The testes are positive in man, guinea-pig, rabbit and mouse. The greatest acid phosphatase content is that in the spermatogonia and the least in the interstitial cells. The prostate is particularly rich in the enzyme, but it is also found in moderate amounts in the lining of the epididymis, seminal vesicle and vas deferens. The female genital tract is positive, the ovary and the lining of the fallopian tube and uterus containing the enzyme. A mod-

erate amount of acid phosphatase was found in the thyroid, parathyroid and anterior lobe of the pituitary.

Discrepancies between the results here recorded and those reported by previous investigators would seem to be explained by the improvements in the technic described above. It is believed that a more complete demonstration of the activity of acid phosphatase is achieved by the present method.

The function of acid phosphatase in the sites described cannot as yet be stated. It is hoped that observations made under a variety of physiological and pathological conditions may provide additional data. However, the localization of the enzyme in individual cells and structures should be of considerable value in attempting to ascribe functions to the enzyme.

As was true of alkaline phosphatase, the neoplastic elements in the tumors examined resembled their cells of origin in acid phosphatase content. For instance, in the oligodendroglioma, and as a rule in the astrocytoma and meningioma as well, only the nuclei were positive. Occasionally the cytoplasm and processes of tumor astrocytes contained the enzyme and, as was noted above, this was rarely true of normal astrocytes. Both cytoplasm and nuclei of the choroidal cells in a papilloma contained acid phosphatase as did normal choroidal cells. In contrast to the paucity of acid phosphatase in the cytoplasm of most meningiomas and its complete absence in some, alkaline phosphatase was present in considerable amounts in most of these neoplasms and was in part correlated with a tendency to calcification. No definite differences in structure that could be related to its absence could be recognized in two of the three meningiomas which contained no acid phosphatase. These two tumors were rich in alkaline phosphatase. One of the meningiomas which contained no alkaline phosphatase was positive for the acid phosphatase. One tumor which was negative for both acid and alkaline phosphatases was an angioblastic meningioma composed almost entirely of tumor vessels and containing relatively few typical arachnoid elements.

Two rather malignant tumors of the nervous system, the glioblastoma and medulloblastoma, were relatively rich in acid phosphatase. Alkaline phosphatase occurred rather irregularly in glioblastomas and was confined principally to astrocytes, whereas acid phosphatase was present abundantly in nearly all the tumor cell types. In the medulloblastoma only the nuclei, however, were positive.

In respect to the homologous reaction of tumor cells and their cells of origin, it is interesting to note that although Gomori¹² found that tumors of the pancreas and ovary were negative, as he found those or-

gans to be, two tumors originating in the bronchi were positive. This is in accord with the positive findings in normal bronchi obtained with the present method.

SUMMARY

1. The histochemical technic of Gomori¹² for demonstrating acid phosphatases in tissues was modified to insure optimal enzyme activity. A variety of substances including enzyme poisons were used to establish the properties of these enzymes in tissue sections.

2. Using this improved technic the distribution of acid phosphatases in normal and neoplastic tissues is described. Acid phosphatase activity was found in nuclei as well as in the cytoplasm of many cells. The nervous system was found to contain large amounts of an acid phosphatase, as did both the male and female genital systems, parts of the digestive, hematopoietic, urinary, and endocrine systems.

3. A series of tumors of the nervous system was studied and the acid phosphatase content of the tumors correlated with the enzyme content of the cell types from which the tumors were derived.

4. The significance of the histochemical technic in relation to function of the enzyme in individual cells is considered.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 44

FIGS. 1 and 2. Guinea-pig. Midbrain. Deep staining of nerve cells and their processes in Figure 1 is the result of their acid phosphatase content. Figure 2 illustrates an adjacent section used as a control, in which the enzyme was inactivated by fluoride. There is no equivalent staining in this section.

FIG. 3. Human cerebrum. Frontal cortex, third cortical layer. The deep staining of some of the pyramidal nerve cells and their processes is an index of their acid phosphatase content.

FIGS. 4 and 5. Guinea-pig. Cranial nerve. Sharp and deep impregnation of axons seen in Figure 4 is an indication of their acid phosphatase content. An adjacent section shown in Figure 5, in which the enzyme has been inactivated by fluoride, reveals no comparable staining.

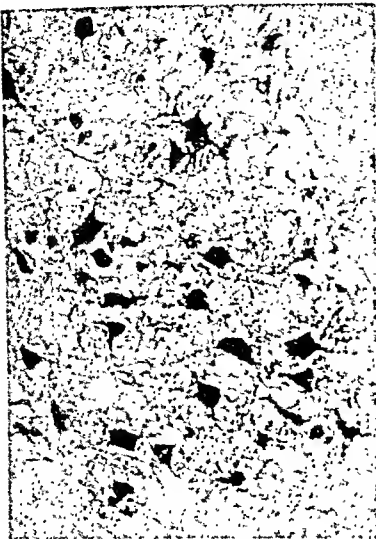
FIG. 6. Guinea-pig. Corpus striatum. The axons show a deep impregnation while chiefly the nuclei are stained in the nerve cells. The dark staining is a measure of the acid phosphatase activity.

FIG. 7. Guinea-pig. Anterior horn of spinal cord. Deep staining of motor ganglion cells demonstrates the degree of acid phosphatase activity.

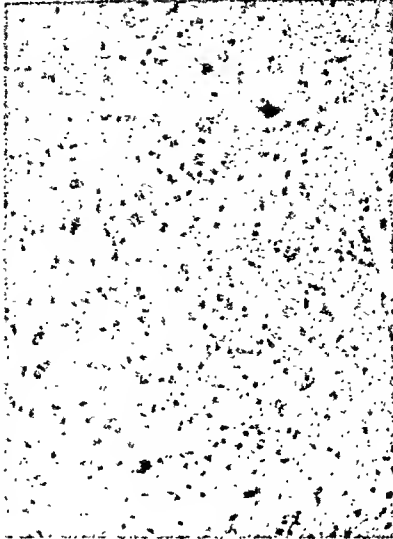
FIG. 8. Oligodendroglioma. Specimen for biopsy of a human brain tumor. The nuclei of the neoplastic oligodendroglia stain deeply while their cytoplasm is unstained, indicating that acid phosphatase is entirely confined to the nuclei.

FIG. 9. Sympathetic ganglion, human. Deep impregnation of nerve cells and axons indicates the presence of acid phosphatase activity.

FIG. 10. Meningioma. Specimen for biopsy of a human intracranial tumor. Only the nuclei of the tumor cells show evidence of acid phosphatase activity.



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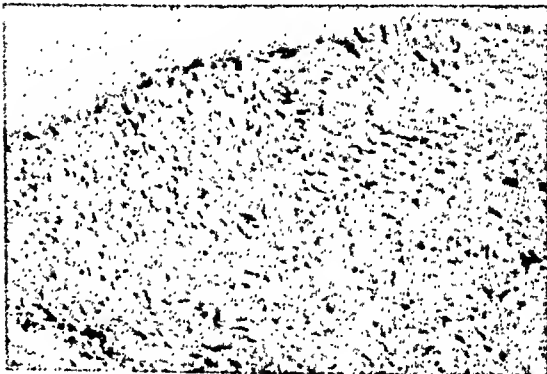
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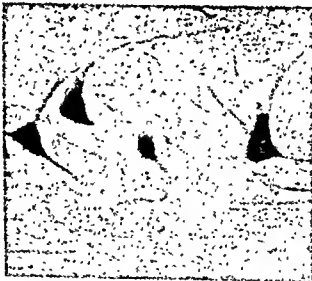
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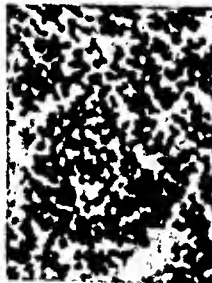
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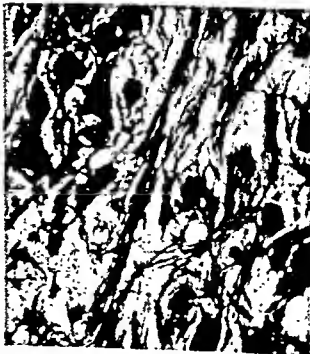
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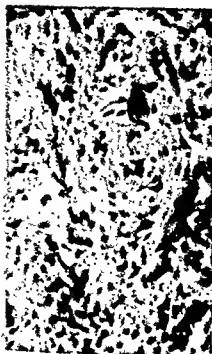
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Wolf, Kabat and Newman

Distribution of Acid Phosphatases

PLATE 45

FIGS. 11 and 12. Heart. Human myocardium. Muscle nuclei and fibers stain deeply in Figure 11, showing that acid phosphatase had been present in them. The lack of similar staining in an adjacent section of the myocardium in Figure 12 is due to the inactivation of the enzyme by fluoride.

FIG. 13. Human prostate. The presence of a considerable amount of acid phosphatase in the lining of the tubules is demonstrated by the deep staining.

FIG. 14. Human liver. The hepatic cells show evidence of their acid phosphatase content by their intense impregnation.

FIG. 15. Guinea-pig. Uterus. Dark staining of the epithelium lining the mucosal glands, of the nuclei of the cells of the stroma, and of the smooth muscle cells is the result of acid phosphatase activity.

FIG. 16. Human pancreas. Impregnation of the acinar and islet cells, the latter more deeply, is a measure of their acid phosphatase content.

FIG. 17. Guinea-pig. Pons. Deep staining of nerve cells and their processes gives evidence of acid phosphatase activity.

FIGS. 18 and 19. Guinea-pig. Lung. The bronchial epithelium, nuclei in general, and the smooth muscle of the wall of the bronchus and that of the artery, in Figure 18, are stained darkly as a result of their acid phosphatase content. The lack of similar staining, in Figure 19, is due to inactivation of the enzyme by fluoride.



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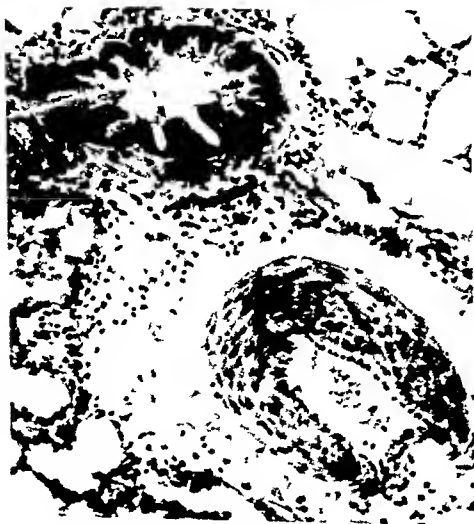
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Distribution of Acid Phosphatases

TUMORS OF DERMAL APPENDAGES *

- I. Tumors of Sebaceous Glands by Shields Warren, M.D., and Wesley N. Warvi, M.D.
 - A. Benign
 - B. Malignant
- II. Tumors of Sweat Glands by Olive Gates, M.D., Shields Warren, M.D., and Wesley N. Warvi, M.D. (*July*)
 - A. Hypertrophy, hyperplasia and metaplasia
 - B. Tumors of true sweat glands
 1. Syringoma
 2. Hydradenoma papilliferum
 3. Hydradenoma
 4. Hydradenoid carcinoma
 - C. Tumors of specialized sweat glands
 1. Ciliary gland
 2. Apocrine gland
 3. Ceruminous gland
 - D. Tumors ascribed to sweat glands
 1. So-called sweat gland carcinoma of breast
 2. Turban tumors
 3. Mixed tumors
- III. Epithelial Cysts and Cystic Tumors of the Skin by Wesley N. Warvi, M.D., and Olive Gates, M.D. (*September*)
 - A. Cysts
 1. Epidermal
 2. Epidermal—traumatic
 3. Sebaceous
 4. Sweat glands
 5. Dermoid
 6. Follicular
 - B. Cystic benign tumors of familial nature, "epithelioma adenoides cysticum"
 - C. Calcified cysts and calcified epithelioma

* The article which follows is the first in a series of three in which tumors of dermal appendages are described. The second and third articles will appear in the July and September numbers, respectively, of this Journal. The general plan of the series is set forth in this outline.

—Editor

TUMORS OF SEBACEOUS GLANDS *

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A. BENIGN

The benign tumorlike enlargements of the sebaceous glands have a histologic appearance which may not differ appreciably from that of the normal gland. There is no clear histologic distinction between so-called adenoma of sebaceous gland and the hyperplastic and hypertrophic glands.⁴⁶ The abnormality is chiefly one of increase in size, number and location of the glands (Fig. 1), with minor aberrations such as absence or atrophy of basal cells, variations in the tendency for the central cells to degenerate, or absence of ducts. Atrophy of the lesions is not uncommon; some may become fibrotic, others may entirely disappear.²⁶

In pathologic enlargement of the sebaceous glands varying degrees of both hypertrophy and hyperplasia are present. Rhinophyma is a diffuse form of excessive local hypertrophy and hyperplasia of sebaceous glands of the nose, sometimes extending onto the cheeks and chin, supposedly a result of chronic inflammation.^{20, 23, 53, 67} The epidermis is thickened and dotted with wide-mouthed ducts leading into enlarged sebaceous lobules full of retained secretion.

Circumscribed lesions have been described according to their clinical characteristics: (a) those occurring in old age, (b) those appearing at birth and at puberty, (c) those associated with other cutaneous and visceral abnormalities forming the syndrome of Pringle's disease.

There is a discrete glandular enlargement sometimes known as "Caspary's sebaceous adenoma" or as senile sebaceous adenoma which occurs in patients after the fourth decade, rarely earlier.^{23, 59} Multiple nodules develop on the exposed parts of the face, especially the forehead. They are asymmetrically placed, small, yellowish, slightly translucent, rather flat and sometimes umbilicated, and have been described as neoplastic⁵⁰ and as hyperplastic.^{27, 35, 70, 73} There is usually an associated dermatitis of the involutional senile type.⁷³

The circumscribed lesion in the young patient is usually considered congenital and is frequently spoken of as a nevus. It is a single plaque-

* Because of the close anatomic relationship of meibomian and sebaceous glands we have considered tumors of these structures as a single group. A review of the tumors of meibomian glands may be found in Scheerer's paper.⁶¹

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like lesion formed of numerous papules. Histologically the normal glandular structure may be slightly altered; atrophy or focal proliferation of basal cells, mild cystic degeneration of the glands and dilatation of the ducts with keratinized material may be present.^{48, 73} Jadassohn³⁸ first reported a form which occurs soon after birth. This is not common.^{44, 45, 60} The naevus epitheliomatosus sebaceus capitis occurs on the scalp often soon after birth.^{29, 54, 72} It is hairless and pitted with the orifices of the ducts. The size is variable and may reach 5 cm. in diameter. One of the few sebaceous adenomas Unna⁶⁹ was willing to accept was a congenital tumor of the scalp reported by Bock.⁷ This had remained pea-sized until old age when it developed into a tumor 8 by 6 by 3 cm.

Many of the glandular enlargements on the face which occur near or at puberty are not symmetrical^{33, 41} and some of them may well represent transitory functional disturbances of the gland.³⁹

There are cases which do not fit into any group, such as those tumors which develop in early adult life, some of which are single, others multiple but asymmetric.^{56, 58} These are probably unusual forms of the acquired senile type or the congenital type. Attempts to classify too precisely on clinical grounds nodules of the skin having similar gross structure have led to confusing distinctions.

An example of this is the controversy as to the status of adenoma sebaceum of the so-called Balzer type. Symmetrically distributed lesions over the nose, cheeks and nasolabial folds and sometimes over the chest usually appear in early childhood or at puberty.²¹ Increase in size may take place several years after the first appearance.¹⁶ They are yellow to pink and sometimes made more conspicuous by telangiectasia of the overlying skin. The arrangement and appearance of the tumors may be identical with those described as Brooke-Fordyce disease. The lesions of two similar cases reported by Balzer and Ménétrier⁵ and Balzer and Grandhomme⁴ as adenoma sebaceum have also been considered as Brooke-Fordyce disease (epithelioma adenoides cysticum). Contemporary criticism is inconclusive.⁶⁹ Ingels'³⁷ recent discussion of the relation between multiple cystic lesions of skin appendages is of interest.

The term adenoma sebaceum is usually associated with the name of Pringle,⁵⁷ who gave particular attention to its histology. Pringle's disease has come to mean a syndrome of multiple congenital abnormalities in which enlargement of sebaceous glands is constant and changes in other organs occur but are variable. The sebaceous growths are disposed symmetrically on the face, especially near the center, and are very small lobular tumors, white to yellowish brown. They appear early

in life and are often familial.³⁰ Von Recklinghausen's disease may be suggested by the presence of cutaneous pigmentation, nevi, papillomas and fibromas in addition to the sebaceous tumors.^{11, 24, 40, 49, 62, 63, 64} In the parenchymatous organs a great variety of mixed tumors, fibromas and cysts may be found.^{19, 51} Mental defects, psychoneurotic symptoms and lesions such as retinal gliomas, tuberous sclerosis and local agenesis of the brain form part of the syndrome in a large proportion of cases.^{8, 9, 10, 12, 15, 17, 22, 32, 36, 47, 52} But in many cases no mental defect is obvious, and some patients are intellectually above the average.^{2, 10, 34, 42, 71} Recent genetic studies by Gunther and Penrose³¹ and Penrose⁵⁵ have shown with a high degree of probability "that a single dominant gene is the main causative factor—and it seems probable that 25–50 per cent of all cases are directly due to a mutation in one or another parent."

We recently had the opportunity to study the findings in an autopsy of a patient suffering from Pringle's disease. The patient was a female, 26 years old, who died of pneumothorax from so-called congenital cystic disease of the lungs. Seborrhic, flatly papular, noninflammatory lesions had been present on her nose and face since infancy and later small papillary fibromata developed over her body. Mental deficiency appeared early and grew progressively worse. Her physical condition had been good until 2 years before death. A moderate degree of dyspnea called attention to bilateral cystic disease of the lungs. Just before death there was marked albuminuria and retention of nonprotein nitrogen. At autopsy, widespread congenital abnormalities were found, most extensive in the lungs. The significant findings were:

1. Hyperplasia and hypertrophy of sebaceous glands
2. Neurofibromata of the skin
3. Congenital cystic disease of the lungs with pneumothorax on the left
4. Tuberous sclerosis associated with cystic softening of the left lenticular nucleus
5. Ovarian leiomyomata
6. Mesenchymal rests of the kidneys and retroperitoneal tissues
7. Recent hemorrhages into renal calyces

The typical sebaceous adenoma of Pringle's disease shows hyperplasia of glands with prominent basal layer of cells. Fibrosis and increased vascularity around the gland may be prominent. These lesions probably belong to the same order of growth as the congenital enlargements known as sebaceous nevi and have no other title to the term adenoma.¹⁴ Usage has nevertheless established them as sebaceous adenomas.

The term adenoma seems to be appropriate for certain tumors of the

sebaceous glands, especially those which are solitary¹⁴ (Fig. 2). Several adenomas have been reported from meibomian gland,^{3, 6, 61, 65} as well as from other locations.^{9, 43, 66} Parreira⁵³ reported 6 cases culled from 1282 cutaneous tumors. Two were quite typical; one other showed an independent basal cell carcinoma superficial to the adenoma and three adenomas had sudoriferous elements as well. Aisu¹ reported a case that is better designated as a hamartoma. Participation of other epithelial structures in formation of sebaceous tumors¹⁸ is not rare in our experience and may be an explanation of some of the controversial cases.

The solitary tumorlike enlargement of the sebaceous gland we accept as adenoma. Grossly it is rounded, nonulcerated, firm, and consists of a circumscribed overgrowth of sebaceous cells producing masses larger than the usual glands and less regular in shape. These enlarged glands frequently lie close to the epidermis rather than in the midcorium (Fig. 1). Typically the picture suggests an expansile growth of the peripheral parts of the gland. As compared to the cells in the center which resemble the normal gland, those at the periphery stain more deeply, partly as a result of less and more finely divided lipoid and partly due to somewhat larger, more hyperchromatic nuclei. Mitotic figures may be absent or fairly numerous (Fig. 2).

REPORTS OF CASES

We report five tumors, none of which was diagnosed clinically as sebaceous adenoma.

Case 1

No. 30-782. C. W. B., male, 63 years of age, presented a slightly elevated nodule 2 cm. in diameter on the left ear. The duration of the tumor was not known, but it had increased in size during the previous 2 months. On examination, it was thought to involve the cartilage and it was therefore excised with the underlying cartilage. It was circumscribed and expansile, preserving the lobulated structure of the normal gland, but the individual lobules measured up to ten times the usual size. Occasional cells at the periphery of the lobule showed mitosis. The tendency of the foam cell of the normal gland to break down into fatty secretion was not noticed. The openings of the glands into the follicles were plugged with keratin and greatly dilated. Keratin was present in the distant ramifications of the dilated duct. It was evident that the gland was not secreting normally although it was actively growing. At the periphery of the primary tumor there was an early basal cell carcinoma that appeared to arise from the skin, and no connection between the two lesions was obvious in numerous sections.

Case 2

No. 35-365. A female, 53 years of age, had a flat papillary lesion on the forehead of unknown duration, measuring 0.7 by 0.3 cm. It consisted of a discrete mass of large sebaceous gland lobules lying in the midcorium. Multiple sections showed narrow, solid, ductlike connections with the skin surface.

Case 3

No. 35-1380. A female had had a verrucous, raised lesion on the right parietal region since childhood, which recently increased in size to measure 5 by 2.5 cm. It was completely excised. Hypertrophied sebaceous lobules several times the normal size made up the tumor. A moderate lymphocytic infiltration was present.

Case 4

No. 40-243. A male, 72 years of age, no history. The lesion measured 0.8 cm. in diameter and was elevated. Enlarged duct stomas were present on the surface. Histologically the tumor was made up of abnormally shaped sebaceous lobules clustered about an irregular arborescence of ducts. There was abnormal keratinization of ducts.

Case 5

No. 48909. A male, 61 years of age, no history. A papillary tumor 0.8 cm. in diameter with a slightly roughened surface was found. The main portion of the lesion was made up of a spherical mass of large atypical sebaceous gland lobules and measured 0.6 cm. in diameter. In the center of this mass there were large, irregular, ductlike structures, some filled with keratin and others with inspissated debris. There was noticeable condensation of connective tissue at the periphery of the lesion.

Malignant proliferation has been reported as frequent.⁶⁸ Two of our sebaceous carcinomas apparently developed from adenomas. Pautrier's⁵⁴ report of 10 per cent of carcinomas in 35 cases of "sebaceous nevus" is unusually high. Malignancy has been described in some cases of rhinophyma⁵³ and senile sebaceous overgrowths.²³

Treatment is by destruction with carbon dioxide snow or electro-desiccation, or by excision.²³

Anomalies of sebaceous glands are not infrequent. Some of them have no clinical significance, such as the one described by Giovannini²⁸ in which sebaceous glands with definite ducts are incorporated in the papillae of hair shafts. Fordyce's disease, on the other hand, merits some interest because of its common occurrence. In this condition yellow punctate lesions are found on the lip and on the buccal mucosa. Fordyce²⁵ noted the frequency of this condition in adults, its familial nature and its varied extent depending on the age at the time of onset. He interpreted the process as downward prolongation of epidermis with accumulation of intracellular fat and some degeneration. The resulting structures have been better described as anomalous hypertrophic sebaceous glandular elements.⁶⁷

SUMMARY

True sebaceous adenomas are rare. We report five cases. Most so-called "adenomas" are instances of hypertrophy or hyperplasia of sebaceous glands.

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B. MALIGNANT

The relationship of carcinoma of sebaceous glands to adenoma and to hyperplasia must be left to conjecture. The fact that many tumors persist unchanged for 10 to 20 years and then grow rapidly and ulcerate suggests malignant change in an adenoma. However, histologic evidence of such transformation is not always reliable.^{4, 9, 10, 28} According to Hagedoorn,^{13, 14} there is evidence that half of the adenomas of meibomian glands become malignant.

The influence of chronic irritation is suggested by the conjunction of carcinoma of sebaceous glands with rhinophyma.³⁴ Twort and Bottomley⁴² found oleic acid very effective in stimulating growth of sebaceous cells and they produced malignant sebaceous adenomas in mice by this means. They suggested that the fatty acid breakdown products of sebaceous secretions, particularly oleic acid, make the cells more sensitive to stimulating influences, such as hormones or chronic irritation, and may be a factor influencing malignant change.

The only carcinoma that can be characterized as sebaceous is one which reproduces at least in some part the characteristics of the normal gland. There is no conclusive evidence that sebaceous carcinoma may be a product of metaplasia of basal cells and keratinized cells unrelated to the sebaceous gland or that sebaceous cells may develop keratinized or nonkeratinized cutaneous carcinomas, although in some instances the histologic picture suggests metamorphosis from one type to another.^{15, 21, 37} This concept of the mutability of cell characteristics of cutaneous epithelium has complicated the study of carcinoma. Unna⁴³ reported one case of sebaceous carcinoma and intimated that the tumor could be recognized only in the early stages by demonstration of its origin from the gland, since he believed that the sebaceous gland structure is almost immediately lost. Masson and G ry²⁸ reported four tumors demonstrating gradations from basal cell carcinoma to completely differentiated sebaceous carcinoma. Grynfeldt¹² described an "epithelioma baso-sebacie" in which both basal and sebaceous elements were present. Duboucher, Montpellier and Cosset's⁷ tumor, a "metatypical mixed sebaceous epithelioma," had a variety of cell types representing all cutaneous epithelial structures. Loos²⁵ reported a basocellular carcinoma of sebaceous gland. Morard³² proposed four subdivisions of the main group of carcinoma of the sebaceous gland: basosebaceous, spinosebaceous, mixed metatypical and sebaceous carcinoma. Such a classification may be useful in emphasizing the variety of cell appearance found in some of these tumors. In some sebaceous carcinomas that we have seen there is a loss of differentiation in parts of the tumor and the cells may resemble keratinized epithelium or

TABLE I
Sebaceous Gland Carcinoma
Reported Cases Not Included by Beach and Severance

Author	Date	Patient		Location	Size and appearance	Duration and symptoms	Treatment and results	Remarks
		Age	Sex					
Unna ⁴³	1896				No data (case no. 37)			
Kren ²⁰	1918	74	M	Face	Pale, red, smooth, and hard; linseed size	6 years	Excised; recurred	From meibomian gland
Komoto ¹⁹	1919	54	M	Left lower eyelid	Walnut size	6 years	Excised; recurred	From meibomian gland
Yataka ⁴⁵	1920	68	F	Eyelid	Hazelnut size	10 years	Excised; recurred twice	From meibomian gland
Akiya ¹	1920	40	F	Left upper eyelid		1 year		From meibomian gland
Masuda ²⁹	1922	69	M	Left upper eyelid		5 years		From meibomian gland
Pereyra ³⁶	1922	58	M	Left lower eyelid	Golden, nodular, hard; egg size	15 months	Excised; recurred	From meibomian gland
Letulle and de Lapersonne ²⁴	1923	75	M	Right lower eyelid	Golden color, hard; hazelnut size	13 years	Excised; recurred twice	From meibomian gland
Matsumoto ³⁰	1925	44	M	Left upper eyelid		3 years	Excised; recurred twice	From meibomian gland
Kitabori ¹⁸	1927	59	M	Left lower eyelid	Egg size	1 year	Excised; recurred	From meibomian gland
Cicconardi ¹⁶	1931	62	F	Scalp	Ulcerated and involved most of scalp	20 years	Excised; recurred	From meibomian gland
Dupuy-Dutemps ⁸	1932	70	M	Eyelid	Large, golden, nodular, hard	Long duration	Excised; recurred three times	Arose from adenoma
Gernez and Gasne ¹⁰	1932	48	M	Right scapular region	Small, ulcerated, hard	7 months		Greater part was sebaceous
Milian and Brunei ³¹	1933	70	M	Nose	Hyperkeratotic, hard surface	20 months		Metastases to angle of jaw
Flarer ⁹	1933	55	F	Left neck	Lobulated, ulcerated; pigeon-egg size	Many months		
Pasca ³⁵	1934	58	F	Right upper lid	Pea size; ulcerated		Excised; rapid recurrence; radiotherapy of slight benefit	From meibomian gland
Charbonnel ⁵	1935	56	F	Popliteal space	In scar of burn received 50 years previously, size of palm of hand; inguinal nodes enlarged			
Parreira ³⁴	1935	73	M	Nose	4 cm., ulcerated, infected	4 years	Excised; rapid recurrence; death by cranial invasion	Generalized metastases at autopsy
Parreira ³⁴	1935		F	Nose	Said to be similar to no. 1			Case of V. Antonia; no information
Loos ²⁵	1936	71	M					Specimen received without information

atypical basal epithelium. It is also true that fatty degeneration of the cells of an epidermoid carcinoma may simulate a sebaceous carcinoma, but rarely to the point of confusion with it.

The incidence of sebaceous carcinoma will be doubtful until it is generally recognized as a distinct entity. Parreira³⁴ gave the incidence of sebaceous carcinomas as 4.6 per cent of all cutaneous tumors. Carcinomas have been reported more often from meibomian than from other sebaceous glands.²² Because of the rather vaguely defined criteria, we feel that conclusions drawn from the literature are of limited value. However, we have tabulated 20 cases of supposed carcinoma of sebaceous gland not included by Beach and Severance² in their recent review of the literature. Certain other cases were omitted because the original publications were not available.^{11, 17, 33, 38, 41}

We have found 29 cases of carcinoma of the sebaceous glands among some 4000 cutaneous carcinomas. One of the tumors came from the anal region of a Great Dane. Five of our tumors, not previously reported, were included in the summary of the literature made by Beach and Severance.² The following discussion is based on our own experience.

There is nothing striking in the gross pathology that would suggest sebaceous carcinoma. The tumor typically develops in the middle or lower corium distinct from overlying epidermis and is often quite discrete although not encapsulated. The presence of sebaceous secretion may give a yellow color to the tumor. It is rather more apt to become infected and have a foul discharge than other carcinomas.

On the other hand, the distinctive histology labels it unmistakably. In most of the examples we have studied, the structure is that of a moderately malignant, locally invasive carcinoma. Both the cells and the pattern of growth closely resemble, or may be indistinguishable from, the normal gland. However, even in the most highly differentiated parts there are certain variations from normal structure. For example, the flattened peripheral cells of the normal gland are not present. Instead the external layer of cells tends to be slightly basophilic and less heavily vacuolated. In one very extensively infiltrating tumor there were normal appearing sebaceous glands, which were, nevertheless, an integral part of the tumor, and, nearby, finely divided strands and masses of cells of typical vacuolated sebaceous type extended widely between striated muscle fibers. However, the majority of tumors in our series have been less differentiated (Figs. 3 and 4). In these there is greater variation in size and shape of cells, the nuclei are intensely hyperchromatic, mitotic figures are more numerous and the lipoid is in finer globules or absent. The cytoplasm may be eosinophilic or

TABLE II
Sebaceous Gland Carcinoma

Number	Age	Sex	Location	Size in cm.	Ulceration	Duration (years)	Treatment	Recurrence	Metastases	Results	Remarks
21-1793							Excision				
22-787	52	F		1			Excision				
23-1088	60	F	Temple	2.5	+	4-5	X-ray, 10/21; radium, 5/22, 6/22, 8/22, 1/23, 2/23, 3/23, 7/23	Never entirely removed		Died from extensive growth of tumor involving half of face	Carcinoma of breast(?) Carcinoma of uterus(?)
23-1089											
S27-581	74	M	Temple	3 x 3	+	10	Diathermy excision; radium seeds to recurrences; radium to enlarged lymph nodes	Twice	Regional nodes(?)	Died 2 yrs. after treatment, cause unknown	
S28-97							Excision				
S28-98		M							Regional node		
S28-391	74	M	Temple				Excision				
29-1704	72	M	Trunk		+	> 1	None		Regional nodes	Died soon after admission; autopsy performed	
30-2855	42	F	Lip	0.5							Congenital(?)
32-559	68	M	Temple	2	+	1	Excision	Once			
32-1637*	62	F	Nose			3	Cautery	Twice		No tumor 3 yrs. later	
32-1719							Excision				
34-1077							Radium				
39-729							X-ray				
34-1931	68	F	Leg			8				Died, cause unknown	Carcinoma of cervix

	M	Eyelid	2-5	+	%	Excision Palliative x-ray 2400 r.		Died 10 weeks after admission No tumor 4 yrs. later No tumor 2 years later	No evidence of recurrence at death Extensive tumor involving upper lid, side of face, malar region
35-1323*	75			+					
37-S-140	70	Face		+					
37-493*	70	Scalp	0.5						
37-1534*		Scalp		+	½				
37-2101	54	Scalp	4	+	4				
37-2553	67	Trunk	7 x 4						
38-S-762	60	Arm	2						
38-1579	58	Scalp	3	+	Life				
39-1264*	63	Trunk	0.5		> 1				
39-3142*	70	Forehead	1	+	1				
39-3296	71	Scalp							
40-782	84	Eyelid							
40-1875	52	Hand	3		4	Excision			
40-2650	66	Lip	2	+	1	Excision			
40-3807	77	Nose			3	Excision			
41-1533		Nose							
41-2363		Arm	3			Excision			
41-4337	Great Dane dog	Anus							

* Mentioned in table by Beach and Severance.²

basophilic and sometimes keratinized. This varied appearance often presents difficulty in diagnosing the tumor from a small specimen. The keratinization may be so marked as to suggest epidermoid carcinoma, although the absence of connection with the overlying epidermis and the architecture are not in keeping with this diagnosis. Conversely, slowly growing epidermoid carcinomas, under certain conditions, contain small fat globules as a product of degeneration, thus simulating the foam cells of sebaceous type. Ordinarily there is little confusion with typical basal cell carcinomas: the cells of sebaceous carcinoma, although sometimes basophilic and without vacuoles, are more rounded, the arrangement of the cells is less compact, peripheral palisading is absent and the architecture is not suggestive of basal cell carcinoma. It cannot be overemphasized that the typical structure of the sebaceous carcinoma is sometimes discernible in only small foci. Recurrent growths may be either more or less differentiated than the primary tumor. The two metastatic growths we have seen resembled the primary tumors.

The tumors usually occur in middle life and there is no significant difference in the incidence with which the sexes are affected. If the numerous carcinomas of the eyelid are excluded, the face and the scalp are involved with almost equal frequency and are the most important sites, but tumors occur on the trunk and extremities as well. Growth is slow, ulceration is late and even very large tumors may still be covered with intact epidermis. But there are exceptions. A few tumors ulcerate early and grow fairly rapidly.

Recurrence and metastasis are frequent problems. There was recurrence in three of our cases. One tumor recurred following excision. Two carcinomas recurred after excision and radiation. Three patients are known to be without recurrence 2, 3 and 4 years after excision of tumors. With one of the tumors which recurred later there were enlarged regional nodes. Proved metastasis to regional nodes occurred in two other cases without recurrence of the primary tumor.

In one, the tumor was a pedunculated fungating mass of friable, necrotic tissue with a foul, musty odor, lying over the fourth to sixth ribs on the right lateral aspect of the chest wall. It was said to have been present several years and had not been treated. Metastases filled the axillary nodes. The tumor and metastases were examined at autopsy. Death was due to renal insufficiency as a result of renal stone and pyelonephritis.

Prognosis is quite good if adequate excision is done at an early stage. Since metastasis is late, with few exceptions excision of recurrences may result in cure.

We have too little information to evaluate methods of treatment. All but three of the tumors in our series were excised. In one case palliative x-ray irradiation was given for a very extensive lesion of the face and the patient died soon after, apparently from the effects of the growth, although no autopsy was performed. Another tumor failed to respond to x-ray irradiation and repeated radium treatments over a period of $1\frac{1}{2}$ years and the patient died as a result of the tumor involving one-half of the face. Another tumor recurred 9 months after diathermy excision but regressed after being treated with two gold seeds of 1.3 mc. each. Enlarged preauricular lymph nodes were similarly treated. This patient, who was 74 years old, died of unknown cause 2 years after the first treatment. Another carcinoma of the nasal septum and upper lip had been present 3 months when it was removed with electrocautery and an unknown amount of radium was administered to the site. There was almost immediate recurrence which was again treated with radium. One and one-half years after the first treatment there was an ulcerated, fungating, infiltrating tumor involving the nasal septum and upper lip. This was excised completely but 2 years later there was a recurrence 1.5 cm. in diameter. This was also excised. The patient, a woman 62 years old, was rather uncoöperative and was not seen again for another 2-year interval when a lesion 2 cm. in diameter was found in the same location. This was given 400 r. of high voltage x-ray irradiation and 1 month later 1000 r. of high voltage x-ray irradiation. After $2\frac{1}{2}$ years there was a recurrence 2.5 cm. in diameter, involving the upper lip and causing fixation in the region of the frenum. A complete excision was again attempted and 3 years after this operation the patient was free from disease.

Although the tumors in our series have been radioresistant, we feel that radiation therapy was not given an adequate test. There is little to be found in the literature bearing on the relative efficacy of surgery and radiation. Surgical excision has been the most frequent form of treatment. Lebensohn²³ reported cure of carcinoma of a meibomian gland by radium. Manganotti²⁷ included carcinomas of the cutaneous appendages in his discussion of radiosensitivity and therapy without any clear-cut conclusions. Magnusson²⁶ stated that all superficial tumors of the skin respond in the same way to radiation regardless of their structure. This opinion is shared with reservations by van der Burg,⁴⁴ but not by Snoke.⁴⁰ Judging by Hintze's¹⁶ report, glandular carcinomas of the skin show the same resistance as adenocarcinomas of most other organs. The effect of x-ray and radium on such carcinomas must be cauterizing rather than selective.³

SUMMARY

Sebaceous gland carcinoma is a pathologic entity, although often confused with basal cell or epidermoid carcinoma. We have encountered 29 cases in our laboratory. The tumor must resemble sebaceous gland in at least some portion. These carcinomas are often resistant to treatment and not infrequently metastasize. Many of them probably arise from benign growths.

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DESCRIPTION OF PLATE

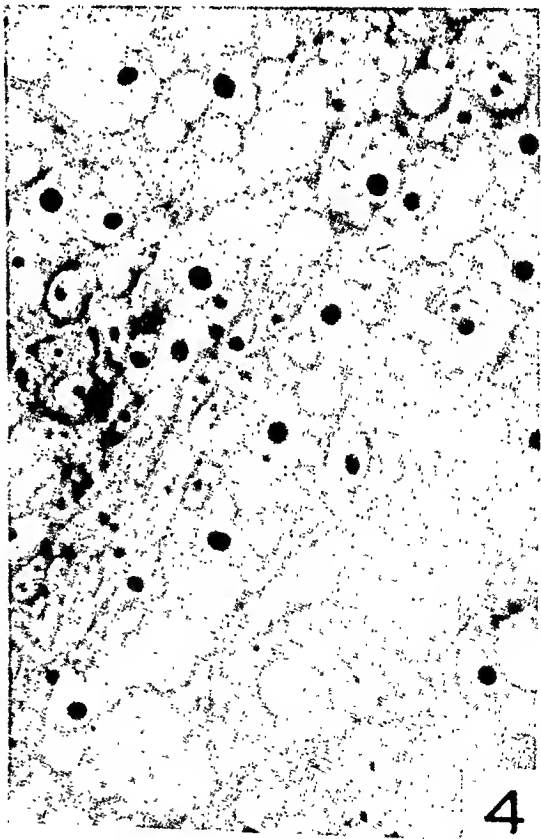
PLATE 46

FIG. 1. Hyperplasia of sebaceous glands. Hematoxylin and eosin stain. $\times 50$.

FIG. 2. Adenoma of sebaceous gland. Hematoxylin and eosin stain. $\times 23$.

FIG. 3. Carcinoma of sebaceous gland. Well-differentiated type. There is invasion about heavily stained striated muscle fiber. Phosphotungstic acid hematoxylin stain. $\times 265$.

FIG. 4. Carcinoma of sebaceous gland. There is variation in cell size, vacuolization of cytoplasm, and dense nuclei. Phosphotungstic acid hematoxylin stain. $\times 900$.



Warren and Warvi

Tumors of Sebaceous Glands

MESOTHELIOMAS OF THE UTERINE AND TUBAL SEROSA AND THE TUNICA VAGINALIS TESTIS

REPORT OF FOUR CASES *

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A degree of temerity is required in attempting to discuss neoplasms arising from endothelial and mesothelial tissues. It has even been maintained that no primary tumor of pleural endothelium or mesothelium has ever been demonstrated.¹ One obstacle in such a study is a certain disagreement as to the meaning of "endothelium" and mesothelium." Histologists are in nearly complete agreement that *endothelium* is the accepted term for the flattened cells lining the lumina of the blood vascular and lymph vascular channels, and that *mesothelium* is to be applied exclusively to the cells lining the serous cavities; *i.e.*, pleura, pericardium, peritoneum and tunica vaginalis testis. Moreover, it is recognized that the vascular channels and the cellular lining of the serous cavities have independent embryological origins and anatomically are not connected.² Yet many writers use the term "endothelioma" for primary tumors of the serous membranes.

In this report, "mesothelioma" is used to identify primary tumors taking origin from the lining cells of the serous membranes. In 37 case reports, in medical literature, of primary tumors of the pleura, pericardium, or peritoneum, 22 are called endotheliomas, 13 are designated as mesotheliomas, and 2 as celotheliomas.

This cursory tabulation of reports of primary serous-membrane tumors from the cumulative index for the past 5 years indicates that a much larger number of tumors regarded as endotheliomas, mesotheliomas, or celotheliomas are found in the pleura than in the other serous membranes; the pericardium coming next in order of incidence, as follows: pleura, 30; pericardium, 5; peritoneum, 2. No case reports were found of tumors so named in the tunica vaginalis. One lymphangioma of the tunica vaginalis is recorded. The great majority of these reported tumors are clinically and morphologically malignant.

The four cases here reported have been encountered recently. They constitute a small group of tumors of obviously similar or identical nature, two occurring in the female pelvis and two in the tunica vaginalis of the testicle. Clinically all of these appeared to be benign in character. The histological pattern is strikingly characteristic, apparently unique and readily recognized microscopically.

* Received for publication, August 17, 1942.

I have failed to find in medical literature any accounts of tumors similar to the one here reported involving the serosa of the uterus. Several reports of tumors of the epididymis and tunica vaginalis with the characteristic histological pattern are available, but these have not been previously considered as mesotheliomas or endotheliomas, and have been variously diagnosed.

REPORTS OF CASES

*Case 1**

H. F. (laboratory no. 936), married, white woman, age 52. Pelvic symptoms led to recognition of a tumor of the uterus. Abdominal supracervical hysterectomy was done. The patient made an uneventful recovery and has remained well.

The specimen consisted of an enlarged uterine body containing a rounded tumor mass about 7 cm. in diameter, intramural in position but extending to the serosa of the uterus. On section the greater part of the tumor appeared grossly to have the structure typical of leiomyoma, being firm and fasciculated. The serous surface presented several clear gelatinous cystic structures about 1 cm. in diameter, the surface between the cysts being somewhat roughened. The cut surface presented a distinct and peculiar zone about 8 mm. in thickness, covering that portion of the tumor immediately beneath the uterine serosa (Fig. 1). This zone appeared more homogenous and lighter in color than the remaining myomatous tumor.

Gross and microscopical examination of the tumor in its relation to the uterus made it clear that the peculiar tissue constituting the superficial zone was not confined to this area but penetrated deeply throughout the myomatous tissue. However, it did not invade the myometrium nor the endometrium. The histological features are described and discussed below. In brief, the structure was adenomatous in appearance.

Case 2†

L. L. (laboratory no. S. J. 735), was a white woman, age 45, who for 3 years had suffered from profuse menstruation, resulting in pronounced secondary anemia. In September, 1941, subtotal hysterectomy was done by abdominal section, including the removal of both tubes and one ovary.

The body of the uterus after removal was moderately enlarged and contained multiple rounded fibromyomatous tumors. The endometrium presented a small (8 mm.), firm polyp, just above the level of the internal os. The right ovary was 3.4 cm. in its greatest diameter and contained small cysts.

* From the service of Dr. W. W. Holly, and Dr. Ralph Crumrine, pathologist.

† From the service of Dr. D. A. Harwood, and Dr. R. H. Osborne, pathologist.

The fallopian tubes were of normal appearance and size, except for the presence of a small, rounded tumor upon the wall of one tube. This was a firm, spherical nodule about 8 mm. in diameter with a granular surface and was almost white in color. Microscopically the sections presented neoplastic tissue with a glandular pattern similar to that seen in case 1. The surface was in places covered by a layer of cuboidal epithelium-like cells.

*Case 3 **

L. W. (laboratory no. W. M. H. 42-360), was a white male, age 66. At examination he presented a mass in the left scrotal sac, which had been first noted 22 years before and was slowly growing. The mass was hard, smooth and of globular shape, and was apparently attached by a narrow isthmus to the lower pole of the testicle, which was otherwise normal.

The tumor was removed surgically. It was found attached to the parietal layer of the tunica vaginalis adjacent to the lower pole of the epididymis. It was free in the tunica vaginalis, which contained about 20 cc. of clear fluid, except for its attachment by a pedicle about 1 cm. in diameter. Its surface was fairly smooth, and its shape was globular, measuring approximately 2.5 cm. in diameter (Fig. 2). About 2½ months later, the operative site was healed, without symptoms or abnormal findings. Microscopically the tumor presented a neoplastic pattern practically identical with that of case 1.

Case 4 †

W. D. (laboratory no. 18-C-42-36), was a white male, age 53. For about 3 years he had noticed a small nodule in the scrotum which was slowly growing and painless. Examination revealed a small, round, hard mass apparently attached to the lower pole of the left epididymis and freely movable within the scrotum.

The tumor was removed under local anesthesia. It was attached by a broad pedicle (one-fourth of its circumference) to the epididymis, and measured 1.7 cm. in diameter. The outer surface was fairly smooth. The cut surface was firm, whitish and somewhat fibrous, and at the periphery had an apparent capsule. Microscopically the structure of this tumor was strikingly similar to that of the neoplastic tissues of the previous cases. Following removal the operative site healed without incident.

HISTOLOGY

As indicated in the preceding brief case reports, these four tumors presented a striking uniformity in structure. The first impression was that of a tumor of adenomatous type. Careful study led to the conviction that the characteristic tumor cells were not epithelial in character, but were mesothelial.

* From the service of Dr. Theodore Bergman, and Dr. R. H. Osborne, pathologist.
† From the service of Dr. C. H. MacKay, and Dr. V. L. Andrews, pathologist.

The glandlike structures varied greatly in size and shape. The cells lining the acini, however, did not have the appearance of true glandular epithelium. They were markedly unequal in size and dissimilar in shape, varying from low, flat plates to a cuboidal or low-columnar form. The flat cell-forms had a tendency to take the "chain" appearance characterizing mesothelial cell membranes (Fig. 3). Many groups of cells were solid, lacking open lumina. A notable cellular characteristic was that a large proportion presented vacuolated cytoplasm, the vacuoles varying greatly in size and giving the cells a "signet-ring" appearance. These vacuoles apparently served as the origin of new glandlike acinar cavities. With the expansion of the cavity, a proliferation of the cell occurred resulting in a new acinus lined by multiple cells. Some of the rounded vacuoles and resulting lumina contained stringy or granular material which, with special stains, gave the tinctorial reaction of mucin (Fig. 4). Staining for fat showed the content of these vacuoles not to be lipid material.

The interstitial tissue framework varied in amount and was largely collagenous fibrous tissue. A moderately rich network of blood vessels was present. A striking feature was the presence of groups of lymphoid cells in certain areas, sometimes so aggregated as to suggest follicular formation (Fig. 5). Special stains for reticulum revealed an abundant network of reticular fibrils intimately related to the epithelium-like cells. Special stains for elastic fibers revealed a moderate amount of elastic tissue in the interstitial trabeculae around the cell groups.

The free surface of the tumor of the tunica vaginalis in case 3 was covered by a rather dense fibrous capsule, but in the other three tumors no definite capsule was present at the serous surface. The findings at the serous surface in the uterine tumor, case 1, were of particular significance. The surface presented multiple papillary and cystlike projections, the cysts being lined by characteristic mesothelial cells. The free surface was covered by mesothelial cells, which were manifestly hypertrophic in many areas, the cells being cuboidal or low-columnar in shape. These surface cells were seen to be continuous with the cells lining the glandlike structures in the body of the tumor through apertures into which the surface cells dipped. This finding is shown clearly in Figures 6 and 7. In the other three tumors the identity of the cell types lining the acini with the cuboidal mesothelial cells upon the tumor surfaces seems obvious. However, the demonstration of the direct connection of the surface mesothelium with the acinar cells is not so clear-cut as in the uterine tumor.

It is my impression, based upon the clinical histories of these cases

as well as the microscopical appearances, that they are essentially benign neoplasms.

COMMENTS

A search of the literature revealed reports,³⁻⁶ several with photomicrographic reproductions, of at least six tumors of the epididymis, of which the histories, gross descriptions and microscopical patterns indicate that they are of the same nature as the tumors here reported. These have been described under various diagnoses. One was called a cavernous lymphangioma. Three were regarded as grade I adenocarcinomas, and two as adenomas of the epididymis.

Oberndorfer³ described his case as a walnut-sized, firm white tumor which protruded into the cavum vaginale, resting on the lower pole of the right testis. Microscopically he described the tumor "meshes" as approximately the size of seminiferous tubules of the testis. He particularly described the numerous groups of lymphocytes scattered throughout the tumor. He regarded the glandlike structure as a lymphangioma.

Thompson,⁴ in his report of 13 tumors of the epididymis, included seven carcinomas. Of these seven, one was designated grade II, two as grade IV, and four were regarded as grade I adenocarcinomas. Of these last four, two were illustrated by photomicrographs which show them to be tumors of the same type as the two tumors of the tunica vaginalis in the present report. A third one was described by Thompson as being identical with the two of which photographs were shown.

DISCUSSION

Assuming, for purposes of discussion, that these tumors constitute a group which has not heretofore been clearly recognized as such, what are the possible histogenic classifications which should be considered?

1. *Epithelial Tumors (Adenomas or Adenocarcinomas)*. The cell morphology fails to correspond to any epithelial type with which I am acquainted. The location, and anatomical and histological relationships are inconsistent with an origin from any recognized normal epithelial structures. If, however, it should be assumed that these tumors are epithelial, are they benign adenomas or adenocarcinomas? The clinical course of all of the cases in my group, as well as those previously described, indicates a benign character. This corresponds to the histological picture, including absence of mitotic activity.

2. *Vascular Endothelial Neoplasms (Angio-endotheliomas)*. Lymphangiomas and hemangiomas are recognized groups of vascular tumors of greater or less cellularity, but in my study of such tumors, structures

of a pattern identical to that of those here described have not been seen. And, further, the demonstrated relationship of the spaces and channels in my present group to the serous surfaces precludes angiomatous character because of the recognized histological independence of the two structures.

3. *Mesonephromas*. Recently attention has been called⁷ to a group of tumors usually involving the ovaries which are regarded as originating from cell-rests arising in the mesonephros and recognized by the presence of structures suggesting imperfect glomeruli and Bowman's capsules. The view is held that their origin is from the embryonic mesonephros which lies in intimate relationship to the developing gonads, and that the presence of their kidneylike structures in the adult ovary is thus explained. It is admitted that there is a superficial similarity between the cellular structures here described and some of the appearances illustrated for the so-called ovarian "mesonephromas." A careful study, however, of the comparative histology fails to show any essential similarity.

4. *Mesotheliomas*. In view of the foregoing considerations, particularly the anatomical location of the tumors in immediate relationship to the serous membranes and the clear-cut continuity of the cells lining the acinuslike spaces with the lining mesothelial cells of the overlying serosa, it is held that these tumors should be denominated *mesotheliomas*.

That each of this small group of tumors was located in direct relationship to the generative organs suggests the possibility that the histogenic factors concerned may be related to the potentialities of the specialized mesothelium of the urogenital ridge, which in the embryo serves as the origin of the gonadal epithelial structures. It will be of interest to know whether tumors of this histological type may be found in the other serous cavities or in portions of the peritoneal cavity more remote from the urogenital ridge.

SUMMARY AND CONCLUSION

Four tumors of markedly similar microscopical structure, located in direct connection with the female or the male generative organs and involving their serous membranes, have been described and the fact pointed out that histologically similar tumors have been previously described, but have been variously classified.

It is concluded that these tumors represent a type not heretofore generally recognized, and that the facts presented justify the view that the characteristic cell structure is mesothelial and that the tumors may properly be considered to be mesotheliomas.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 47

- FIG. 1. Case 1. A section of the tumor of the uterus showing a light colored zone at the serous surface. The peripheral zone is mesothelial neoplastic tissue. The deeper portions are composed of interlacing myoma and mesothelioma. $\times 134$.
- FIG. 2. Case 3. The tumor of the tunica vaginalis showing a free rounded surface enfolded by the tunica, to which it was attached by a large pedicle. $\times 134$.
- FIG. 3. Case 4. Mesothelial cells surround glandlike spaces. The largest lumen is lined on one side by "chainlike" cells. Adjacent is a lumen surrounded by cells in multiple layers. Several of these cells present a "signet-ring" appearance. $\times 315$.
- FIG. 4. Case 3. Large spaces are lined by greatly flattened cells. Three collections of mucinlike material gave a characteristic coloring. Hoyer's stain. $\times 190$.



Evans

Mesotheliomas

PLATE 48

FIG. 5. Case 3. Characteristic tumor pattern under low magnification showing abundant interstitial collagenous tissue and a collection of lymphocytes. $\times 60$.

FIG. 6. Case 1. The surface of the uterine tumor covered by cuboidal mesothelial cells which are continuous through surface apertures with similar cells lining communicating spaces. $\times 120$.

FIG. 7. Case 1. The communication of glandlike structure with surface mesothelium. The stroma near the surface contains many lymphocytes. $\times 140$.



Evans

Mesotheliomas

MYOEPITHELIAL PROLIFERATIONS IN THE HUMAN BREAST*

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The myoepithelial cell of the human breast is a smooth muscle cell which is epithelial by origin and remains on the "epithelial" side of the basement membrane. Myoepithelial cells have been described in the apocrine skin glands, in the mammary gland, in the glands of the eyelids and in the salivary glands on numerous occasions by European investigators. These are cited by Hoepke,¹ Hamperl² and Schultz.³

If one considers the mammary gland as a specialized skin gland, the presence of myoepithelial cells in this structure is more easily accepted, since histologists agree on their presence in apocrine skin glands. In a 9 mm. human embryo one can recognize an epithelial thickening forming a ridge which extends from the neck region to the groin. This has been termed the "mamillary line." Along this line the epithelial anlage of the breast is differentiated and the breast gland takes form by bud proliferation of the epithelial cells into the dermis and subdermis. The solid cell cords develop lumina and undergo cellular differentiation. The cells forming the lumen remain cuboidal and retain characteristics of their epithelial ancestry. However, the cells nearer the basement membrane become elongated and develop delicate fibrils in the cytoplasm. Such cells possess the morphology of smooth muscle cells and form what is called the myoepithelium. This is quite generally accepted, and at present there is no doubt that such cells do exist in the normal human breast (Eggeling⁴).

NORMAL MYOEPITHELIAL CELLS

In the breast the myoepithelial cells are arranged about the ducts, especially the smaller ducts that lead away from the lobules. These cells are the elongated contractile elements which are found between the epithelial cells and the basement membrane. Apocrine glands of the skin have an almost uninterrupted layer of myoepithelial cells, according to Kölliker.⁵ In the eccrine glands there are spaces between the individual cells. However, the same author described thin, intercellular bridges between the epithelium and myoepithelium of both. In the breast the myoepithelial cells are isolated and appear in a proportion of about one myoepithelial cell to every six or seven true epithelial cells. The myoepithelial cells are arranged spirally about the lobular

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ducts. Less characteristically these cells may be found around acini and larger ducts. The individual cells are elongated, with a rather pale cytoplasm in which can be seen distinct, delicate fibrils as found in ordinary smooth muscle. The nucleus is rod-shaped, oval, or spindle in shape with a dense, granular chromatin material. The long axis of the cell is parallel to the basement membrane on which it appears to lie (Fig. 1). On cross section the cell frequently has a typical triangular outline, and, when crowded, the nucleus also may be triangular in shape. The base of the triangle rests on the basement membrane while the apex points toward the lumen of the duct (Fig. 2). Such cells when vesicular in nature are called basket-cells (Korbzellen). During proliferation these cells may lose their proximity to the basement membrane, but do not exceed its confines, remaining on the epithelial side.

Myoepithelium can be demonstrated in the male breast of gynecomastia when the ducts are developed. In the developed breast of the female little difficulty is encountered in its identification. In the breast in pregnancy or lactation these elements are partially obscured by the epithelial hyperplasia, but can be found on very careful scrutiny. In regard to malignant lesions of the breast, myoepithelial elements were not found in ductal carcinomas, scirrhous carcinomas, medullary carcinomas, or in malignant Paget's disease. However, the breast tissue distant from the malignant new-growth did possess myoepithelium. Myoepithelial cells are absent in breasts showing universal atrophy.

IDENTIFICATION OF THE MYOEPITHELIAL CELLS

If one keeps in mind the normal position, distribution and morphology of these cells, little difficulty will be encountered in identifying them. Moreover, if one avails himself of the special staining procedures he will find a specific reaction with some dyes.

In hematoxylin and eosin preparations the myoepithelial cells have bluish black, granular nuclei and a tapering, rather large cell body with a reddish cytoplasm containing longitudinal fibrils. They are usually quite distinct from the epithelial cells except when the latter are crowded or distorted by mechanical factors, in which case the two may be confused. However, study of the cytoplasm, particularly by means of special stains, serves to distinguish them.

In sections prepared with van Gieson's stain myoepithelial cells appear brownish yellow and are usually outstanding. In addition, the van Gieson stain brings out the periductal connective tissue and demonstrates the position and course of the basement membrane. Cellular accumulations of lesser density are yellowish in color.

Probably the most specialized stain for myoepithelial cells is erythro-

sin-saffron as devised by Masson.⁶ In this stain, myoepithelial cells possess a rust-red or bright red cytoplasmic substance, the shade of red depending upon the compactness of the cells.

For demonstration of the basement membrane a silver impregnation procedure serves best. A modification of the Foote method or the original Wilder method is found to be satisfactory. Demonstration of an intact basement membrane separating myoepithelial cells and the true epithelial elements from fibrous stroma or mesodermal derivatives is imperative.

The material for study is best obtained from fresh surgical specimens as soon as possible after removal. The specimen is placed immediately in Bouin's fluid for about 12 to 18 hours and then into 80 per cent alcohol. Bouin's solution was found to be the most satisfactory because it produces the least distortion and allows for good selective staining. Autopsy material may also be used but the length of time the tissue has been "dead" may produce changes which make it more difficult to study the cells accurately. However, material obtained soon after death can be fixed in the Bouin's fluid just as is the surgical material, and it has been found quite satisfactory.

PROLIFERATION OF THE MYOEPITHELIAL CELLS

The most interesting and significant study in regard to the myoepithelial cells is concerned with their proliferations. The myoepithelial cells of both the apocrine skin glands and of the mammary gland have the property of proliferation. Myoepithelial tumors of sudoriferous glands have been described by several European investigators as cited by Sheldon.⁷ This author reported three myoepithelial tumors of the sweat glands, two of which he considered malignant.

The proliferation of myoepithelial cells usually accompanies certain other changes in the breast. Most significantly, such proliferations are found in mastopathia cystica, fibroadenomas, and glandular atrophy with hyalinization of the fibrous stroma.

Myoepithelial proliferations of the mammary gland have been reported by Günther,⁸ who described the changes and also cited several other European investigators. Masson⁹ gave space to myoepithelial proliferations, but this has not been carried on in standard textbooks. However, Hamperl² was the first to lay emphasis on myoepithelial proliferations, particularly in their relation to other lesions of the breast. He described such proliferations with special emphasis on chronic cystic mastitis and stressed the relationship of myoepithelial cells to mixed tumors of the mammary gland of the dog. Fibroleiomyoma of the breast is considered a hypothetical lesion by Foote.¹⁰ Contrary to this,

Strong¹¹ and Melnick¹² each reported a case. In these instances, however, the tumor was thought to arise from the musculature of the blood vessels rather than from the myoepithelium. American literature is devoid of any references to myoepithelial proliferations of the mammary gland, in so far as could be determined.

Mastopathia Cystica

In breast tissue showing mastopathia cystica there is considerable myoepithelial proliferation which at times is so pronounced that the differential diagnosis of the lesion becomes very important. In this series 30 cases were studied; 8 cases showed myoepithelial proliferation of varying degree. These were found in persons between the ages of 50 and 70 years. Usually the proliferation takes place in the smaller ducts and their extensions within the lobules, and in such instances the myoepithelial proliferation is principally into the lumen of the duct. The earliest stage of this process is distinctly pictured in Figure 3. In this photomicrograph there can be seen six myoepithelial cells standing perpendicular to the basement membrane. The epithelial cells thereby form a small tuft which projects into the lumen. Normally the myoepithelial cells on cross section of a duct or gland have their longitudinal axes approximately parallel to the basement membrane. Evidently, then, the change in direction of the long axes of the nuclei may in this case be interpreted as one of proliferation, particularly since one can see the grouping of the cells and the progress of the cell mass toward the lumen. In some of the cystic spaces, and at times in the larger ducts in such cases, one can find a thickening of the lining cells, and this thickening can be demonstrated to consist principally of proliferated myoepithelial elements.

In Figure 4 the wall of the duct reveals a thickening of the epithelium. This portion is three to four times as thick as the remainder. Many of the cells found in this area have elongated, granular, very dark nuclei and fibrillated cytoplasm. Such cells are bright red in color in erythrosin-saffron preparations. Other cells exhibit more oval, pale, vesicular nuclei, characteristic of true ductal epithelial cells. The myoepithelial proliferation is often accompanied by an epithelial proliferation as well, and sometimes this is so far advanced that it produces actual intraluminal papillomas. On examination of such areas it is found that the papillomas are made up of two types of cells: the usual cuboidal or polyhedral type with an oval or round vesicular nucleus, and the elongated, dark spindle-shaped cells. Figure 5 shows an intraductal papilloma in which dark, elongated myoepithelial cells are the predominating elements, but there are also rounded, pale epithelial

cells, particularly about the periphery. Such proliferations are described by Hamperl² as epi-myoepithelial. With special stains these are found to be outstanding and no difficulty is encountered in identifying them. In such instances, with the silver impregnation methods, one can demonstrate argentophilic fibers which extend into the papillary structure, and with these particular fibers the myoepithelial cells appear to be closely associated. If the two elements are not recognized, the differences in cell morphology and intensity of staining reactions may suggest a malignant change (metaplasia) in what is really a benign papilloma. The true nature of the morphologic differences becomes apparent when silver impregnation methods reveal an intact basement membrane and special stains separate the myoepithelial cells from the epithelial cells by color differentiation as well as morphologic characteristics.

Fibroadenomata

In fibroadenomata of the breast, myoepithelial cells frequently participate in the formation of the tumor. Of 29 such cases, including fibroadenomas in the male breast, there were found 8 cases showing myoepithelial proliferation. The individuals in this group were principally between the ages of 20 to 40 years. Proliferations in this tumor of the breast, however, are frequently found to be extraluminal; that is, growth occurs away from the regular parenchyma with penetration of the stroma. In such cases there is a formation of nests or buds of proliferating myoepithelial cells which grow into the stroma. At times the proliferation is quite dense, and a very solid appearing mass of elongated, hyperchromatic nuclei is formed.

Examination of less densely packed myoepithelial proliferations shows the presence of small, round, somewhat vesicular nuclei which sometimes outline a clear space, so as to form a small lumen. These latter cells are really epithelial cells which are associated with the myoepithelial proliferation and which tend to form the acinar or glandular elements, outside of which are the myoepithelial cells. Many irregular acini are seen in Figure 6. One large area is taken up by the very cellular tumor. In this area of cellular proliferation there are found great numbers of elongated, dark, granular nuclei. Usually these are closely related to the fine, threadlike argentophilic fibers that course through the tumor. The acini are lined by pale, cuboidal cells surrounded by the myoepithelial cells. Such a unit of epithelial and myoepithelial cells is separated from others by fine, threadlike, silver-impregnated fibers.

These changes, of course, represent the advanced myoepithelial proliferations. A stage of proliferation in relation to fibroadenoma of a less marked degree is shown in Figure 7. In this area the extraluminal

myoepithelial proliferations have thus far retained their characteristic morphology and have not replaced the glandular portions of the organ to any great extent.

Fibrosis

In certain breasts, particularly those undergoing senile involution, there is found a great deal of hyalinized connective tissue stroma containing myoepithelial nests. This picture may at first glance be confusing. In spite of the fact that myoepithelial proliferations are frequently accompanied by some degree of epithelial proliferation as described above, there are found numerous nests of densely packed, hyperchromatic myoepithelial cells without any demonstrable epithelial cells being present. These nests may be large enough to take up the entire high-power microscopic field, or there may be only several closely packed hyperchromatic cells completely surrounded by a very dense hyalinized fibrous stroma.

The pathogenesis of such a change can best be appreciated after a study of the illustrations. In Figure 8 there is also some myoepithelial proliferation in the lobular duct, but this change does not take place in all instances. The lobular duct here shows a marked thickening of its wall in the upper portion of the figure with a complete absence of epithelial cells. In conjunction with this the acini are quite scarce. The left lowermost acinus shows a partial replacement of its epithelium by myoepithelium while the cell mass in contact with this acinus represents a second acinus in which the true epithelial cells are completely replaced by a proliferation of myoepithelial cells. In such instances one is able to demonstrate by silver impregnation methods an intact basement membrane which limits the cell proliferation from the surrounding fibrous stroma. It seems that in such abundant myoepithelial proliferation the epithelial glandular structures disappear, resulting in the formation of nests of the hyperchromatic, densely packed spindle cells (myoepithelial) in a dense fibrous and, at times, hyalinized stroma.

Figure 9 illustrates a process similar to that demonstrated in Figure 8. Here, however, there are isolated groups of myoepithelial cells separated one from another by connective tissue stroma, and scattered throughout this area are distorted acini. The stroma surrounding this particular remnant of the lobule is seen to be very dense and relatively acellular, and the intralobular connective tissue is similar. There are a few, irregular, isolated acini of relatively vesicular, round nuclei. In close approximation to these there are elongated and irregular nests of very dark staining, closely packed nuclei. With careful study these can be demonstrated as myoepithelial cells, as revealed by their special staining characteristics. It is quite apparent that the acini have no

normal histologic relationship, being separated from each other by dense nests of myoepithelial cells and wide bands of stromal tissue. A very significant point in the differential diagnosis of such findings is brought out in Figure 10. This high-power magnification shows small nests of irregular, markedly hyperchromatic nuclei lying in small clefts between the dense layers of stromal tissue. The similarity of this appearance to that of scirrhous carcinoma should be plainly evident.

DISCUSSION

From the word "myoepithelial" it is apparent that the original intention was to convey the impression that these cells are muscle-epithelial cells. This had its conception in the fact that morphologically they resemble smooth muscle cells, but are more intimately associated with the usual epithelium of the glandular or functioning part of the organ; that is, these cells by position should be epithelial in nature, but by shape and structure are indistinguishable from smooth muscle cells. Consideration of their embryology leads to the conclusion that they are epithelial in origin and remain akin by position to the epithelial elements of the breast gland.

Explanations of the morphology and of the function of myoepithelial cells are not readily apparent. No definite function has been attributed to them. Much speculation has taken account of their morphologic similarity to smooth muscle and thus assigned to them the possible functions of support to the ducts or of aid in the emptying of the glands. It might be more advantageous to consider these cells in the light of their epithelial ancestry and to attribute to them some function associated with epithelial tissue. It is quite evident that they do not have a visible secretion product like the epithelial cells of the breast. Furthermore, rarely do they come in contact with the secretion within the ducts. One may postulate, therefore, that perhaps these myoepithelial cells act as receptors rather than excretors. They may be the post to which the endocrines hitch, or perhaps have an affinity for internal secretions and later deliver the same to the epithelial cells. An internal secretion (renin) has been ascribed to the eosinophilic granulations in the afibrillar myoblast of the juxtaglomerular apparatus of the kidney as described by Goormaghtigh¹³ and Dunihue.¹⁴

At present, however, it is more important to recognize myoepithelial proliferations than to ascribe to them a specific function. In regard to the mammary gland such proliferations have been found to be entirely benign. Kölliker⁵ stated that myoepithelium has the faculty of very readily changing into epithelial cells and assuming the cuboidal morphology. This represents only a "slight aberration" as these cells are

originally epithelial in origin. As such, therefore, they usually remain benign. On the other hand, Hamperl² traced the origin of mixed tumors of the canine breast to myoepithelium. Allen¹⁵ believed in a similar occurrence but gave the epithelium and not the myoepithelium as the origin. In these instances the tumors are more apt to be of the malignant type since the myoepithelial cells in such cases are "totipotent." Certainly in such cases there is less differentiation, and malignant changes naturally would be more frequent. A malignant myoepithelioma was reported by Gaudier, Grandclaude and Lambret.¹⁶ This impression was based on cytology alone. The tumor was encapsulated grossly and was clinically benign. Therefore, it is dubious that the tumor was malignant in the usual sense of the word.

Myoepithelial proliferations may be confused with true malignant changes. As found in cystic mastopathia, proliferations of the myoepithelium frequently consist of irregular thickening of the ductal epithelium, intraluminal papillomatous formations, or proliferating buds of epithelium-like cells penetrating into the stroma. In myoepithelial proliferations in lesions known as fibroadenomatosis there is a prominent stromal infiltration by hyperchromatic, elongated cells. In addition to such cells, the usual epithelial cells are at times found forming small, irregular acini, but these structures are always sharply differentiated from the true stromal tissue by the presence of an intact basement membrane. In hyalinized fibrosis of the breast there are found nests of myoepithelial cells which have survived. Such cells are usually irregular in outline, elongated and hyperchromatic. The nests of these cells are also irregular and are found in the clefts of a very dense connective tissue. In all of these cases, therefore, the elongated, hyperchromatic type of myoepithelial cell, which is found penetrating into the stroma and in isolated nests in the dense connective tissue stroma, may erroneously be interpreted as an aberrant form of the usual epithelial cell. Such aberrations usually suggest the possibility of malignancy. Here, however, they do not indicate malignancy, and such cells may be recognized as myoepithelial if their morphology is closely scrutinized and special stains are used. Such proliferations are always enclosed by an intact membrane which separates them from the mesodermal derivatives. Such lesions have been diagnosed as metaplasia, as precancerous, or as malignant "degeneration." This is evident if one considers those patients with so-called carcinosarcoma (Saphir and Vass¹⁷), microscopically diagnosed as such, who live in good health for many years. From a study of photomicrographs in such cases and from the clinical courses of the patients, it may be concluded that some of these tumors

were myoepithelial in nature. In another article, Saphir and Parker¹⁸ described an intracystic papilloma classified as group III papilloma or "transitional cell type." Such a lesion possesses elongated, spindle-shaped cells, and "may possess an inert degree of malignancy." However, they add that with simple mastectomy the prognosis is good. In the light of the study here reported one might interpret the "transitional cell" papilloma as a benign myoepithelial papilloma. Furthermore, under the title of "Borderline Breast Tumors," Bloodgood¹⁹ presented cases which lived in good health for long periods even though the lesion was suspected of being malignant. Upon comparing his photomicrographs with those presented here, the similarity becomes apparent. Bloodgood, however, did not describe these proliferations as myoepithelial in origin.

For these reasons it is very important in the study of neoplasms of the breast to identify myoepithelial tumors and to allot to such their good prognosis. It is also important not to confuse the myoepithelial tumors with malignant transformations of intraductal papillomas or with carcinosarcoma, scirrhous carcinoma, or malignant transformation in so-called fibroadenomatosis.

SUMMARY

1. Myoepithelial cells of the mammary gland possess the faculty of proliferation, either alone or in conjunction with the usual epithelium, especially in breasts showing mastopathia cystica and fibroadenomatosis.

2. Myoepithelial cells have the power of survival and proliferation in senile involution and fibrosis of the breast.

3. In so far as is now known, proliferations of the myoepithelial cells are benign as long as they retain the characteristics of their epithelial ancestry but may become malignant when forming derivatives of the type usually ascribed to mesoderm.

4. Borderline or suspicious breast lesions should be carefully studied for the presence of myoepithelial elements. If the proliferations are found to be myoepithelial, such lesions are benign and should be distinguished as such.

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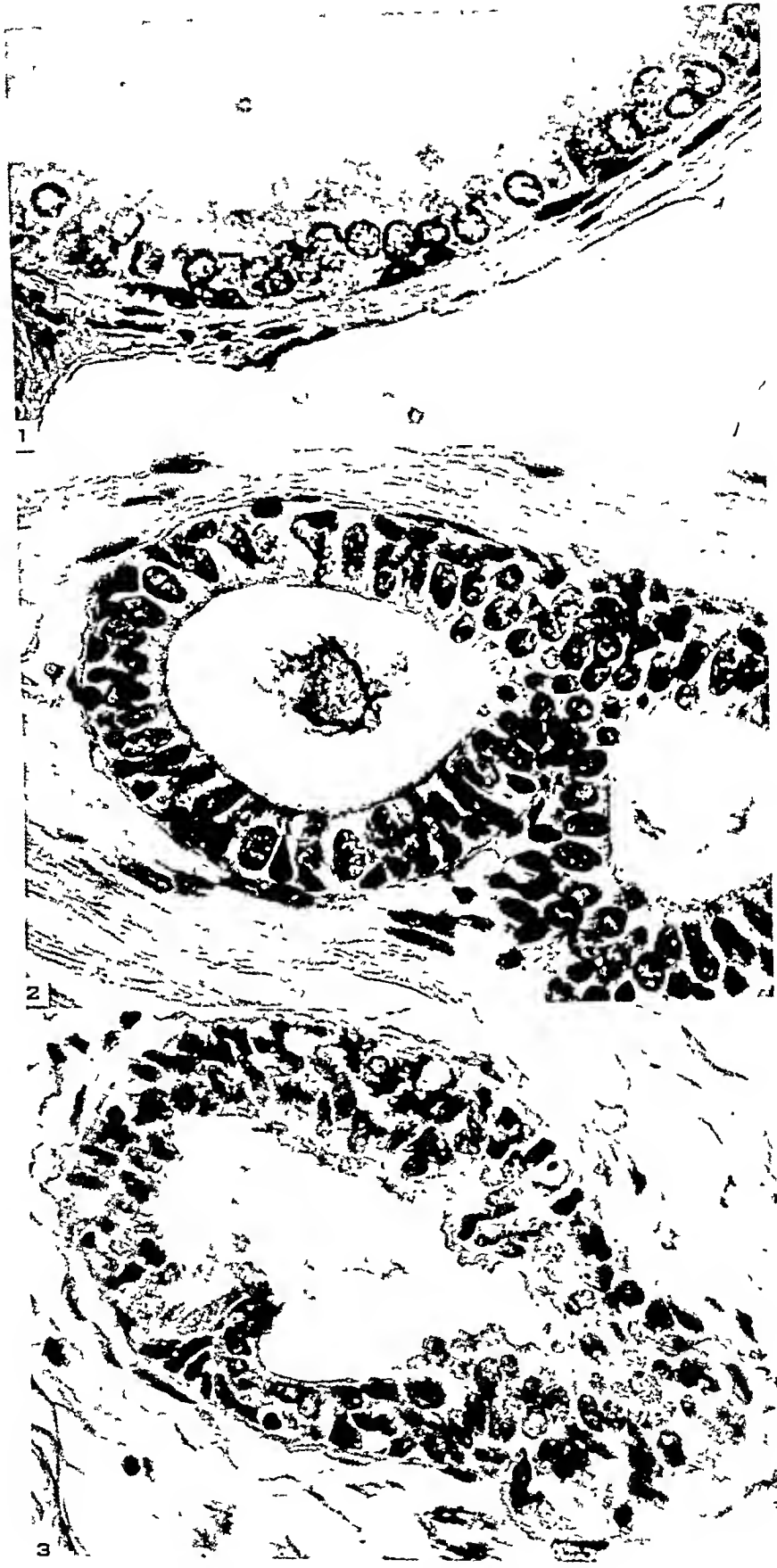
DESCRIPTION OF PLATES

PLATE 49

FIG. 1. Adenoma of the breast from a woman, 60 years old. The longitudinal, spindle-shaped dark nuclei at the base of the epithelial cells and in close proximity to the basement membrane belong to the myoepithelial cells. Hematoxylin and eosin stain. $\times 585$.

FIG. 2. Fibrocystic disease of the breast in a colored female, 55 years old. In this illustration the myoepithelial cells are seen in cross section, thus demonstrating their characteristic triangular shape when so cut. Myoepithelial cells are more abundant than usual. Hematoxylin and eosin stain. $\times 585$.

FIG. 3. A cross section of a small duct from the breast of a woman, 67 years old, with mastopathia cystica and Paget's disease. At the base of the small cellular tuft which protrudes into the lumen from the lower left wall, the elongated dark nuclei standing on end belong to the myoepithelial cells. This is the beginning of an epi-myoepithelial intraductal papilloma. Hematoxylin and eosin stain. $\times 585$.



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Myoepithelial Proliferations in the Human Breast

PLATE 50

FIG. 4. Fibrocystic disease of the breast in a colored female, 52 years old. The illustration includes a portion of the wall of a large duct with stasis and dilatation. In the portion of the thickened epithelium the elongated, deeply stained nuclei, which are here perpendicular to the basement membrane, belong to myoepithelial cells. The cytoplasm of these cells clearly shows fibrils. The basement membrane was demonstrated to be intact. Hematoxylin and eosin stain. $\times 655$.

FIG. 5. Fibrous mastopathy from a woman, 30 years old. An epi-myoepithelial proliferation has produced a papilloma within a duct. The cells composing the papilloma are chiefly myoepithelial, as is shown by the dark, elongated nuclei. There is an occasional spherical, vesicular epithelial nucleus. Hematoxylin and eosin stain. $\times 75$.

FIG. 6. Fibroadenoma of the breast in a middle-aged woman. The entire field is a tumor mass of myoepithelial cells accompanying which are fewer epithelial cells. The irregularly outlined, small, clear spaces are actually acini formed by distorted epithelial cells. Otherwise the cells are myoepithelial. Hematoxylin and eosin stain. $\times 75$.

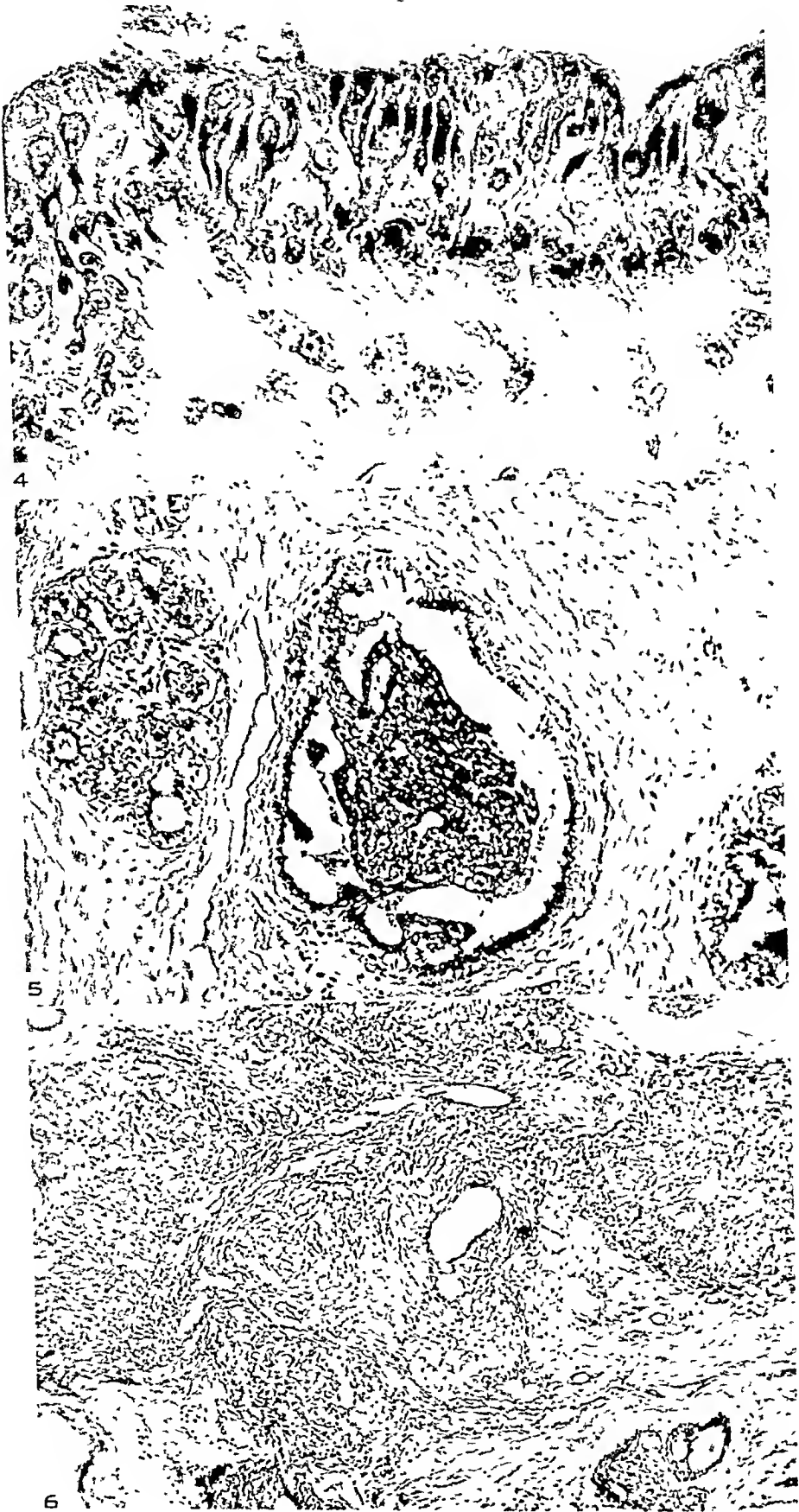
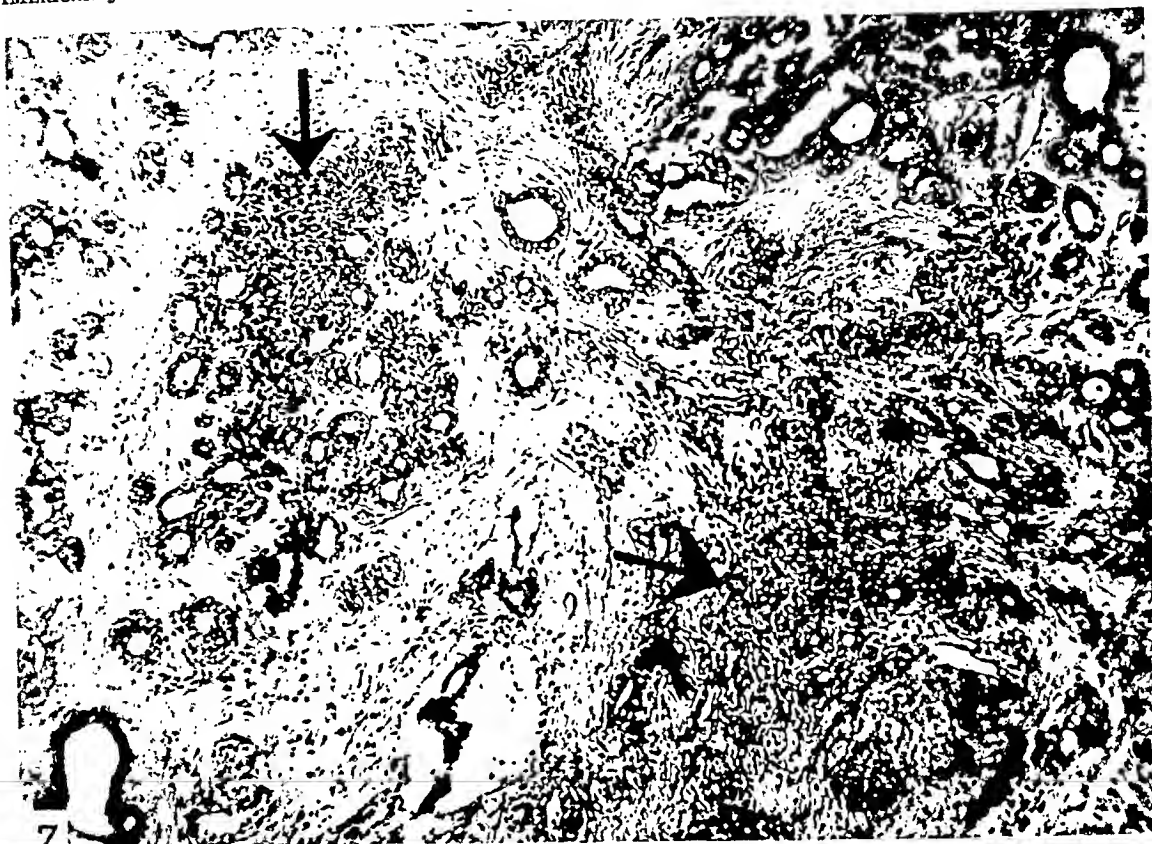


PLATE 51

FIG. 7. This field was made from the breast of a colored woman, 28 years of age. Upon this a microscopic diagnosis of adenofibroma had been made. Here there are shown extensive, yet distinct, myoepithelial proliferations in the stromal tissue from which they are separated by the usual basement membrane, now distorted and tortuous but intact throughout. Hematoxylin and eosin stain. $\times 90$.

FIG. 8. Fibrosis of the breast with dense and relatively acellular stroma. Only the remnants of a lobule remain. The upper portion of the lobular duct reveals a significant myoepithelial proliferation. The dark mass of cells above the lowermost acinus represents a second acinus entirely replaced by myoepithelial cells. Hematoxylin and eosin stain. $\times 90$.



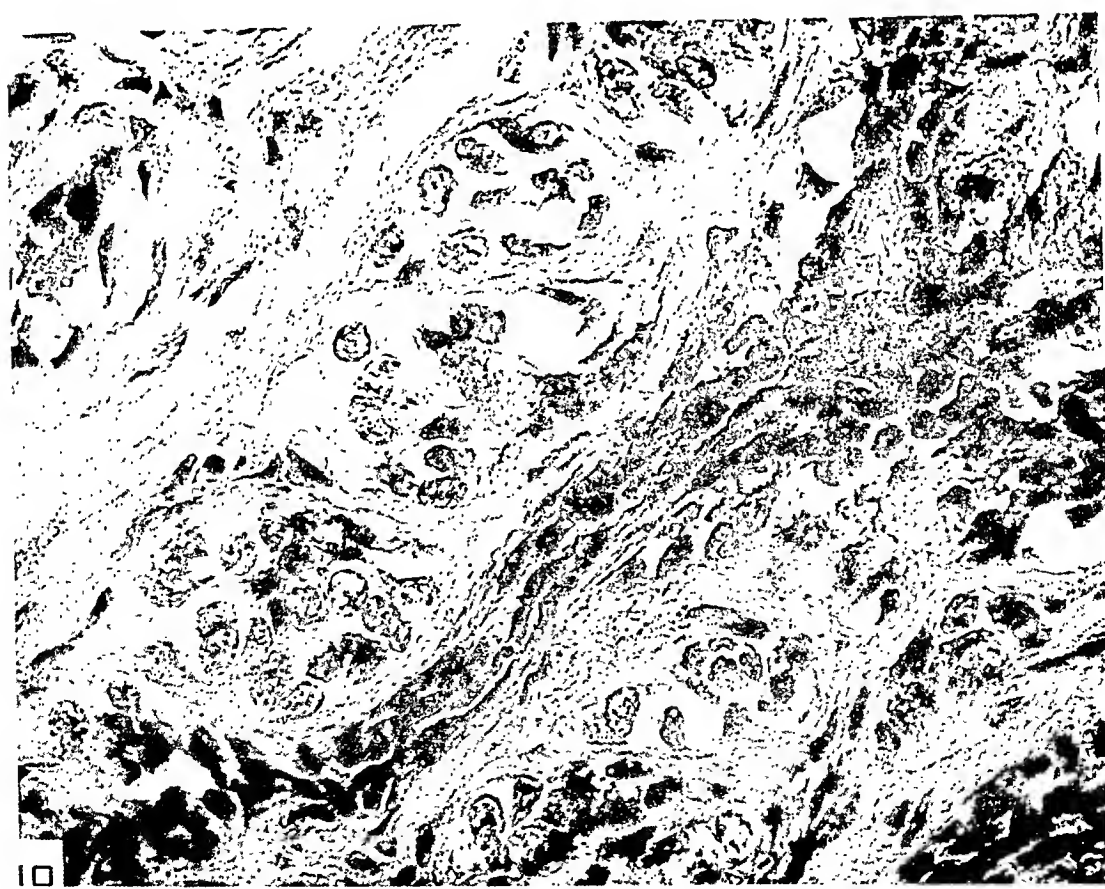
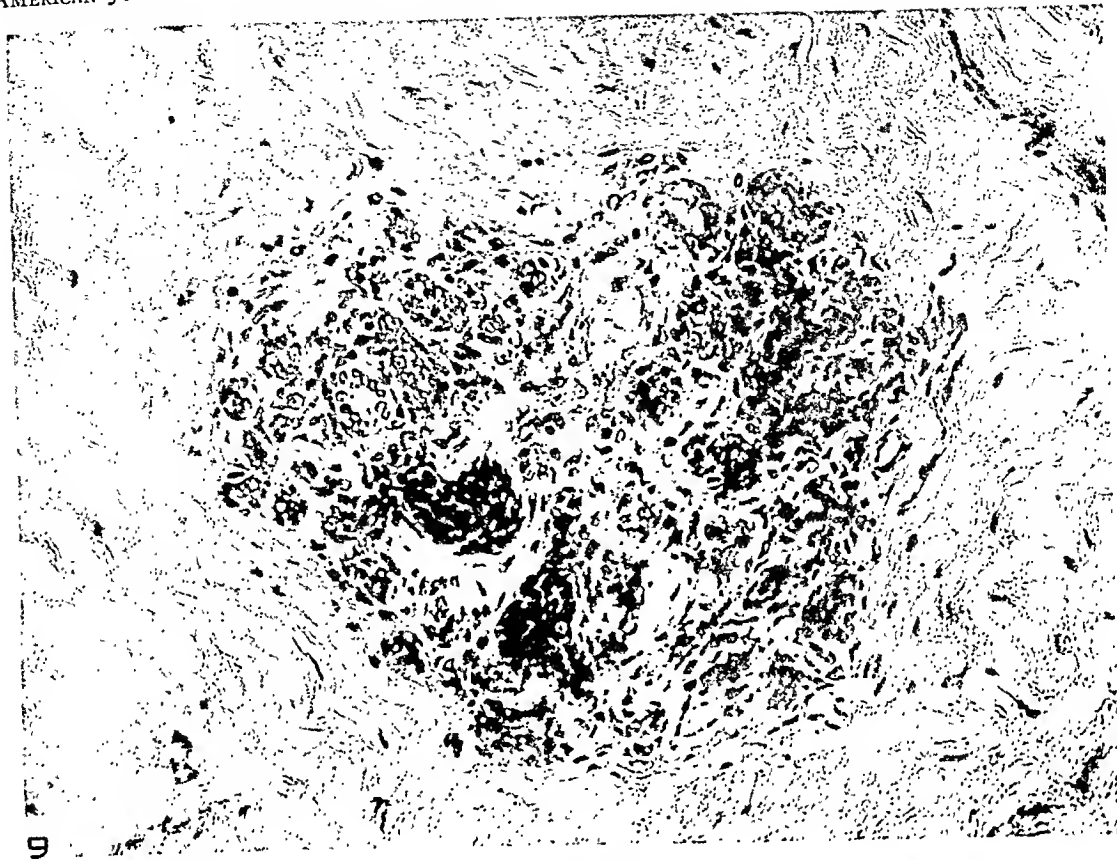
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Myoepithelial Proliferations in the Human Breast

PLATE 52

FIG 9. Intralobular fibrosis and epithelial atrophy of the mammary gland. Irregular nests of elongated hyperchromatic cells have replaced much of the original lobule. These cells are myoepithelial. Hematoxylin and eosin stain. $\times 140$.

FIG. 10. This illustration demonstrates the difference in morphology between acinar epithelium and an elongated group of myoepithelial cells. Such hyperchromatic, elongated, and at times irregular cells may be confused with scirrhous carcinoma when they are found in fibrous stroma after total loss of epithelial cells. Hematoxylin and eosin stain. $\times 785$.



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Myoepithelial Proliferations in the Human Breast

THE STOMACH IN PERNICIOUS ANEMIA *

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In view of the recent evidence¹ suggesting, contrary to previous reports, that in man the site of production of "intrinsic factor" is the fundus and body of the stomach, it is pertinent to reconsider the gastric changes in patients with pernicious anemia. Several reports²⁻⁵ have described acceptably the anatomical changes in the stomach in this disease, but since the number of cases adequately studied has been small, the findings in a group of six autopsied cases of pernicious anemia in which the stomach has been studied anatomically at Stanford University have been reviewed. Two of the patients in this series differ from the reported cases in that they had continuous successful liver therapy for many years.

Table I summarizes the principal significant clinical features of the cases which are here reported. The diagnosis of pernicious anemia seems reliable in each, although the two patients (cases 1 and 2) who were under successful therapy for 13 and 10 years, respectively, had no anemia at the time of death. These, however, had had characteristic episodes in which there was rapid disappearance of anemia after the institution of liver therapy, although there is no record of reticulocyte counts from either patient. In cases 3 and 4 the patients, who had had therapy but died before the anemia disappeared, had distinct increases in blood reticulocytes following liver therapy. The fifth patient (case 5) had a macrocytic hyperchromic anemia, leukopenia, absent tendon reflexes in the legs and a history of a similar anemia 3 years previously which was diagnosed pernicious anemia and which disappeared after liver administration. During the recurrence of the anemia, liver therapy was given for only 3 days and no significant reticulocyte response had occurred at the time of death. The spinal cord showed slight demyelination in the dorsal columns, the liver contained moderate amounts of hemosiderin, and even after the 3 days of concentrated liver therapy the bone marrow obtained at autopsy was hyperplastic and contained a few cells identified as hemoglobinized megaloblasts by Dr. Harry Wyckoff of the Laboratory of Clinical Pathology. The remaining patient (case 6) was not recognized as having pernicious anemia before death and no specific therapy was administered. This diagnosis, made at autopsy, is supported by the presence of hematogenous pigmentation

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TABLE I
Clinical Data

Case	Age	Sex	Duration of treatment	Lowest red blood cell count	Reticulocyte rise	Signs of spinal cord injury	Free HCl in gastric juice after histamine
				(million)	(% r.b.c.)		
1 (OD 412)	78	M	13 years	3.1*	No record	—	o
2 (9C 177)	80	M	10 years	1.8*	No record	—	o
3 (41R-106)	42	M	2 months	2.8	12	+	o
4 (1D 86)	74	F	20 days	1.2	6	+	o
5 (1D 52)	62	F	3 days†	2.6	1.3	+	No examination
6 (ODF 7)	74	F	o	1.6	o	—	No examination

* Red blood cell count normal at death.

† Three years before death a similar anemia disappeared following liver administration.

of many of the organs and a very hyperplastic bone marrow which contained cells resembling megaloblasts.

METHODS

The stomachs to be described were all obtained at autopsy within 9 hours of the time of death. Two specimens which had remained within the body for longer than 6 hours showed superficial post-mortem digestion in some areas, but even here most of the mucosa was intact. In the other four cases the autopsy was performed between 2 and 4 hours after death and the stomachs were only slightly altered by post-mortem changes. It is felt that these changes have not significantly obscured the mucosal structure in any of the cases.

The stomachs were stretched on a board before fixation so that all mucosal folds were flattened, and, after fixation for 24 to 48 hours in a 4 per cent solution of formaldehyde, strips of mucosa were dissected from the underlying muscularis along the entire length of the greater and lesser curvatures. These were rolled up like fire hose and sections were cut after the rolls were embedded in paraffin. This procedure insures sectioning of all parts of the mucosa in a plane perpendicular to the surface and prevents fallacious interpretations of the thickness of the mucosa from tangential sections. The histological descriptions to follow were derived from examination of sections stained with hematoxylin and eosin and with Giemsa's stain.

FINDINGS

The dissection was accomplished easily. There was no unusual adhesion of the mucosa to the remainder of the stomach wall in any case. An outstanding characteristic of the stomachs was the marked alteration in the mucosa of the fundus and body, which I shall refer to as the fundic zone, in contrast to the relative freedom from abnormalities in the pyloric portion. This has already been emphasized by Meulengracht.⁵ There were slight alterations in the pyloric zone in several

instances; two showed unusual numbers of cells in the interstitial tissue; in three there were scattered Russell bodies; and in two (cases 4 and 5) there were single, small, protruding mucosal nodules in this region. In cases 1 and 3 the pyloric zone was considered normal throughout and, except for the presence of the polypoid nodules, the changes of the pyloric zone in the other cases were not distinguishable from those occurring in many persons of comparable ages.

In the fundic zone the changes were extensive and severe. The mucosa was only about half the thickness of that in the pyloric zone. Even in the gross specimens this difference was apparent, producing a fairly sharp distinction between the zones (Fig. 1). The abnormal thinness was not due to post-mortem changes, but was a manifestation of a completely abnormal type of mucosa in the fundic area, in which the normal specific cell types (parietal and chief cells) were absent. The mucosal glands (Fig. 2) were shorter, less numerous and more tortuous than those of the normal fundic region. The arrangement was irregular and some glands were separated from one another by loose connective tissue. The deeper lining cells were cuboidal and fairly uniform, but were faintly stained and had no distinctive morphological characteristics. Some glands had formed small cysts, but these were not numerous in any case. Scattered through the abnormal mucosa in all stomachs were easily demonstrable, and sometimes very numerous, coarse, deeply stained glands showing structural features characteristic of mucosal glands in the small intestine (Fig. 4). Some of these atypical structures occurred singly and some were in groups which had completely replaced other glands. These glands of intestinal type contained goblet cells, and Paneth cells with prominent eosinophilic cytoplasmic granules were usually prominent in the basal portions. In case 1 similar granules were also present in the cells of small glands which were tortuous and occurred in well defined but not encapsulated clusters (Fig. 5). It will be noted that this is one of the cases treated successfully for many years. However, case 2, which also received prolonged treatment, did not present this appearance. In four of the stomachs there were considerable numbers of interstitial cells resembling lymphocytes and plasma cells among the abnormal mucosal glands, but in cases 4 and 6 these were not numerous. Similarly, although Russell bodies were abundant in the fundic zone in three cases, they were practically absent in cases 1, 3 and 4. None of the findings varied in consistent relationship to the known duration of the disease or of the therapy. The loss of specific cell types in the mucosa of the fundic zone was complete in all except case 1, in which a few small groups of atypical glands containing a few cells resembling parietal cells were present in the sections taken

from the upper portion of the lesser curvature. No chief cells were seen and no parietal cells were found in any other portion of this stomach.

The stomach from one case of nontropical sprue was examined. This patient had been studied carefully at Lane Hospital in 1928 when he had had a severe anemia, diarrhea and normal gastric acidity. The papillae of the tongue were moderately atrophic but at no time were there any neurological changes. When liver therapy was instituted there was an increase in blood reticulocytes to 16.3 per cent of the total number of erythrocytes. This was followed by a complete remission of the disease, which did not return during 2 years of observation while the patient continued to eat about one-half pound of liver three times a week. After December, 1930, he disappeared for 10 years and was next seen in an almost moribund state with a hemoglobin determination of 12 per cent and a red cell count of 600,000. In spite of attempted therapy he died after 26 hours. Many organs showed hemosiderin deposits which were particularly prominent in the liver where there was extensive diffuse scattering of fine granules of iron-containing pigment, not only in the Kupffer cells but also very prominently in the hepatic cells. In the bone marrow were accumulated large numbers of cells morphologically like the megaloblasts of pernicious anemia.

The stomach, removed 3 hours after death, showed none of the changes which characterized the stomachs of the above-described cases of pernicious anemia (Fig. 6). This is in accord with published reports of the demonstration of normal amounts of free acid in the gastric juice from patients with sprue, but does not support the view (Olleros⁶ and others) that some degree of "gastritis" is present in this disease.*

DISCUSSION

Some doubt has been expressed⁸⁻¹¹ whether all cases of pernicious anemia have characteristic gastric lesions, and there is little question that diseases such as sprue and infestation with *Diphyllobothrium latum* may lead to the development of a similar anemia without evidence of gastric disease. However, the gastric changes in cases of pernicious anemia, such as those here reported, are sufficiently alike and characteristic so that they may be considered as a group. Changes of so-called "chronic gastritis" are frequent in stomachs from patients who have not had pernicious anemia (Konjetzny¹² and others), but although these have some qualitative resemblance to the lesions in the

* Studies of pepsin secretion in this patient showed unusually low values which have been reported in case Ga in a series studied by Pollard and Bloomfield.⁷ The gastric mucosa at autopsy contained abundant chief cells, but they were less intensely stained than usual and the staining reaction was irregular.

cases of pernicious anemia, the extent of the changes and the distribution are different. Post-mortem study in this laboratory of 175 stomachs from routine autopsies by the method outlined above has shown frequent abnormalities of the mucosa of the pyloric zone, but in only four instances were changes in the fundic zone sufficiently pronounced to suggest the lesion in pernicious anemia. Even in these, scattered specific cells were present, distinguishing the stomachs from those of the cases of pernicious anemia. Usually in the absence of pernicious anemia, gastric mucosal changes are limited to the pyloric zone, or are most pronounced in this region. The lesion in pernicious anemia, therefore, may be regarded as probably different from the mucosal change which occurs in many stomachs with advancing age or with diseases other than pernicious anemia.

The cause of the stomach disorder in pernicious anemia cannot yet be pointed out. Evidence for an hereditary influence is convincing,¹³ but this might lie in a predisposition of the mucosa to injury rather than in a congenital malformation. No instance of this type of gastric lesion in an embryo or child has been reported, suggesting that the change in cases of pernicious anemia is an acquired one.

It is unlikely that the lesion is a result of anemia, since achlorhydria has been observed to precede the anemia, sometimes by a period of years, and reports of return of free hydrochloric acid to the stomach after cure of the anemia are rare. The persistence of a practically normal gastric mucosa in some cases of sprue, such as the instance here reported, in spite of the presence of severe pernicious anemia, suggests not only that the anemia itself is not a significant etiological factor, but also that the factors directly causing the anemia do not produce the gastric lesions. The lack of relationship between the amount of treatment and the appearance of the mucosa in the cases here reported does not support the view^{14, 15} that treatment causes disappearance of the "gastritis." The changes sometimes observed by gastroscopists during treatment of pernicious anemia might be due to growth of replacement epithelium, but the histological and functional evidence indicates that a return to a normal mucosal type does not occur.

Inflammation may be part of the process in the stomach, as cellular infiltration of the mucosa is present in some cases, but inflammation alone does not explain satisfactorily all of the observed changes. No significant relationship between the degree of cellular infiltration and the duration of the disease has been noted in the cases presented here, and there was no extensive fibrosis of the mucosa which in all parts could be dissected free from the underlying tissues very easily, in contrast to the firmly adherent mucosa in the zone of inflammation which

borders chronic ulcers. The localization of the process to the fundic portion of the stomach is not readily explained by the concept that the process is primarily inflammatory.

The irregularity of the gastric mucosa in the altered areas and the presence of atypical gland types suggest that some epithelial proliferation had occurred. The frequent presence of a few glands of intestinal type in the gastric mucosa of many people, particularly in older age groups and in association with other mucosal changes, indicates that this type of growth occurs readily in the stomach, perhaps accompanying a number of different types of disease. The frequency of this phenomenon in cases of ulcer and carcinoma of the stomach has been repeatedly emphasized.^{12, 16, 17}

A possible cause of the gastric mucosal changes in cases of pernicious anemia might be some sort of selective massive destruction of the parietal and chief cells with relatively slight injury of the less differentiated cells. If such an injury were followed by limited repair associated with mild lymphoid cell infiltration, changes like those in the stomachs of patients with pernicious anemia might be produced. The appearance in the cases here described is reminiscent of the selective injury to specific epithelial cells in massive toxic necrosis of the liver. If such an episode is survived, hepatic cells may be completely removed from large portions of the liver while less differentiated cells forming bile ducts remain and proliferate. There may be relatively little newly formed fibrous tissue or evidence of inflammation. A comparable process in the gastric mucosa should be expected to affect principally the most differentiated elements—the parietal and chief cells—leaving the pyloric zone relatively unaffected. Such damage might appear only in unusually susceptible individuals in the same way that liver necrosis occurs only occasionally after cinchophen administration, or that bone marrow injury develops after chemotherapy only in a few presumably hypersensitive individuals.

SUMMARY

In six cases of pernicious anemia the stomach showed almost complete replacement of the normal mucosal glands of the fundic type by abnormal, less differentiated glands. The pyloric zone was only slightly altered. No relationship could be found between the appearance of the stomach and the duration of the disease or of the treatment. The stomach from a well-studied case of long-standing sprue with fatal macrocytic anemia showed no comparable changes. The gastric lesions in the cases of pernicious anemia are different from those accompanying other diseases and it is suggested that they may represent a specific

change, perhaps the result of massive destruction of the highly differentiated parietal and chief cells.

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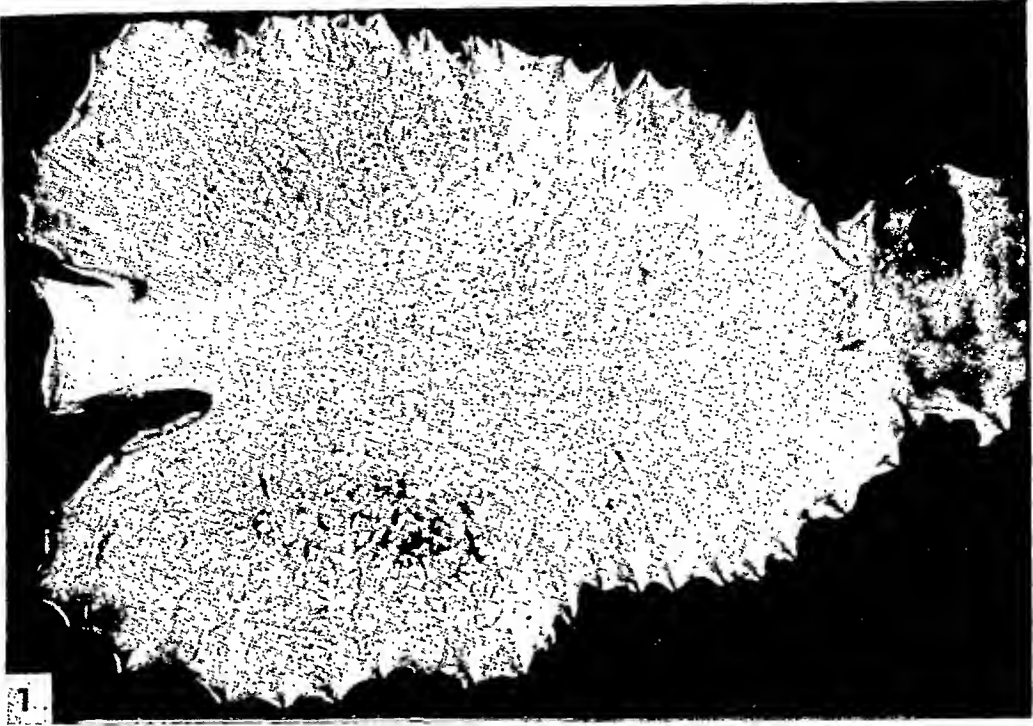
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 53

- FIG. 1. Stomach from case 5, opened along the greater curvature, showing a very thin fundic zone with easily visible submucosal vessels, contrasted with the pyloric zone of normal thickness except for a single, small polypoid nodule. One-third natural size.
- FIG. 2. Section of the fundic portion of the gastric mucosa of case 4 showing abnormal architecture with irregularly arranged, small, tortuous glands entirely devoid of parietal and chief cells. Hematoxylin and eosin stain. $\times 92$.
- FIG. 3. Section of the normal pyloric portion of the gastric mucosa from case 4. Hematoxylin and eosin stain. $\times 92$.



Cox

The Stomach in Pernicious Anem

PLATE 54

- FIG. 4. Section of the fundic portion of the gastric mucosa of case 1 showing interstitial cells in moderate numbers among the abnormal glands, and two darkly stained glands of intestinal type. Hematoxylin and eosin stain. $\times 92$.
- FIG. 5. Section of the fundic portion of the gastric mucosa from case 1 showing great irregularity in structure, with a localized collection of atypical small glands. Hematoxylin and eosin stain. $\times 92$.
- FIG. 6. Section of the normal fundic portion of the gastric mucosa from a case of sprue with fatal macrocytic anemia. Hematoxylin and eosin stain. $\times 92$.



4



5



6

THE DEVELOPMENT OF THE LARVAE OF TRICHINELLA SPIRALIS IN ROLLER TUBE TISSUE CULTURES *

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The desirability of culturing the helminth parasites of vertebrates *in vitro* has been repeatedly emphasized. Hoepli, Feng and Chu (1938) reviewed this problem and concluded that while various adult worms could be kept alive in sterile artificial media for long periods of time, in no case had marked growth and tissue differentiation of larvae been obtained in cultures. Since that time several workers have reported progress, particularly those working with strigeid metacercariae (Ferguson, 1940). However, to date, although Glaser and Stoll (1938) were able to culture the free-living stages of *Haemonchus contortus* and Ackert, Todd and Tanner (1938) were able to obtain an increase in the size of immature *Ascaridia lineata* which were obtained from the intestines of chickens, it has not been possible to obtain sexual differentiation of the parasitic stages of the nematodes of vertebrates. McCoy (1936) attempted to grow trichinella larvae in abnormal environments, pointing out that due to lack of host specificity, rapid growth to maturity, and the ease with which sterile larvae could be obtained, this parasite should prove to be a favorable species for such study. No development occurred in McCoy's Maitland tissue cultures, or in the lumina of nonpregnant rat uteri, or in the amniotic sacs of dead rat embryos, but a small number of larvae developed to sexual maturity in living chick embryos and in the amniotic sacs of living rat embryos.

The present paper reports experiments in which an attempt was made to obtain development of trichinella larvae in roller tube tissue cultures. As far as can be determined, the only previous application of this particular technic to the culture of helminth parasites is that of Mendelsohn (1935) who kept larvae of *Taenia crassicolis* alive for 35 days, but was unable to obtain significant developmental changes.

MATERIAL AND METHODS

Isolation of Larvae

Trichinella larvae were obtained by peptic digestion of stock mice which had been infected from 5 weeks to 6 months before use. The carcasses were skinned, the feet and head cut off and the viscera removed.

* Study initiated with the aid of the George Cheyne Shattuck Memorial Fellowship, Harvard Medical School.

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Gross fecal contamination was avoided by tying off the esophagus and rectum before removal. The carcasses were then washed in cold running water and passed through a meat grinder. In early experiments the trichinous material was digested in battery jars and the larvae concentrated by sedimentation. Later, a modified Baermann apparatus similar to that described by Hobmaier and Meyer (1937) was used. This proved to be a simple technic for obtaining viable larvae that were relatively free from contamination. The meat grinder, Baermann apparatus, glassware and instruments were sterilized by autoclaving before each use. The digestion mixture routinely consisted of 4 gm. of pepsin, 5 gm. of sodium chloride and 9 cc. of hydrochloric acid (sp. gr., 1.19) in a liter of tap water. Digestion was carried out at 37° C. for a period of 6 to 8 hours; longer periods of digestion were found to decrease the number of viable larvae.

Sterilization of Larvae

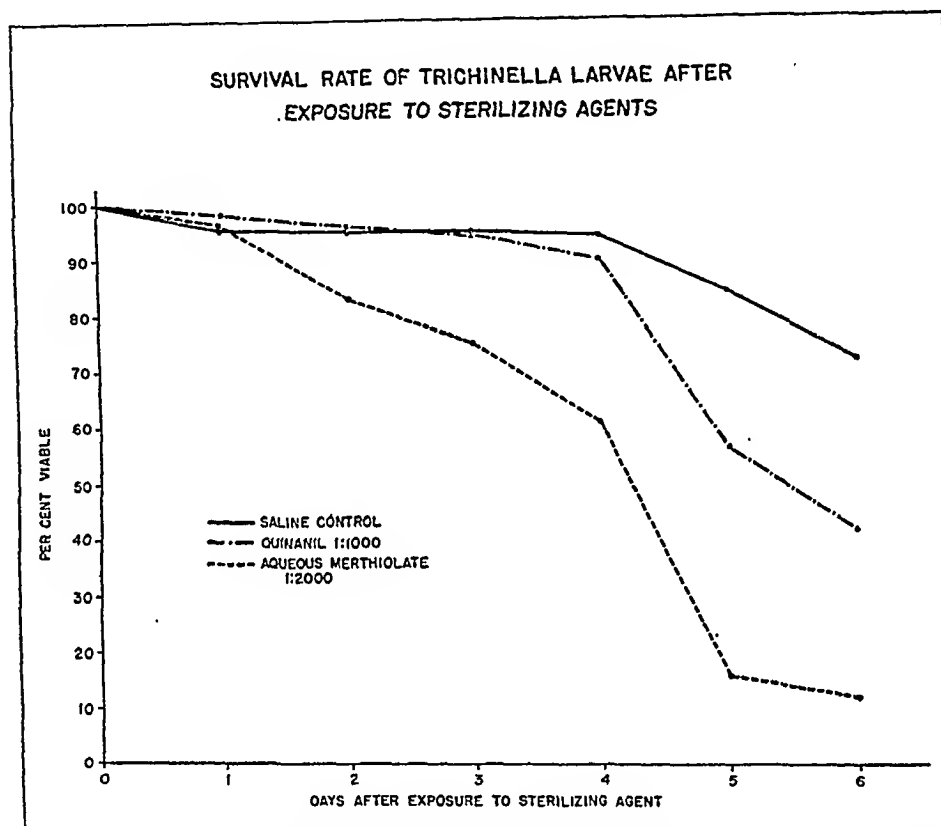
Several methods of sterilizing larvae were used. Routinely, the procedures of "sterilization" and washing were carried out in 50 cc. centrifuge tubes. The larvae were introduced into 25 cc. of the sterilizing agent with a sterile Pasteur pipette, agitated for 2 minutes and then allowed to settle for 3 minutes. Following this they were washed by transfer through five tubes, each of which contained 25 cc. of normal saline solution; sterile pipettes were used for each transfer and the larvae were left 5 minutes in each tube.

In the early studies, a 1:2000 solution of aqueous merthiolate, buffered with 0.07 per cent borax, as recommended by McCoy (1936), was used as a sterilizing agent. Although McCoy demonstrated that a small proportion of larvae so treated possessed the ability to develop to maturity, as the present study progressed and complete development in tissue cultures was not obtained, it seemed advisable to determine whether the sterilizing agents employed had any latent deleterious effect. At the same time the possibility of using other methods of sterilization was investigated. Glaser and Stoll (1940) used sodium hypochlorite solutions for sterilizing and exsheathing nematode larvae. Boxhall, Hapold and Lloyd (1934) found quinamil* to be an effective bactericidal agent in the isolation of a flagellate from fly intestines, and therefore its use was suggested.

Simple *in vitro* experiments were set up to determine the longevity of larvae after sterilization. A typical protocol follows:

* Quinamil is the trade name for 2(p-dimethyl-amino-anil) 6(methyl-quinolene methochloride) and is produced by The British Drug Houses, Ltd., London.

Sterility Experiment No. 6. Following digestion, 100 active larvae were picked up with a mouth pipette, using a dissecting microscope, and placed in each of three Wassermann tubes containing, respectively, 5 cc. of normal saline solution, 5 cc. of a 1:2000 solution of aqueous merthiolate buffered with 0.07 per cent borax and 5 cc. of a 1:1000 normal saline solution of quinanil. The larvae were left in the sterilizing solutions for 5 minutes. They were then washed by transferring



Text-Figure 1. Graphic tabulation of the survival rate of the larvae of *Trichinella spiralis* after exposure to 1:1000 quinanil and 1:2000 aqueous merthiolate solutions, compared to a control group treated with normal saline solution.

each lot of larvae with a sterile Pasteur pipette through five tubes, each of which contained 5 cc. of normal saline solution; the larvae were left 5 minutes in each tube. Following washing, each lot was placed in 5 cc. of normal saline solution in a Wassermann tube and was incubated at 37° C. Each day the larvae were transferred to a Syracuse watch glass, and the live larvae counted and returned to the tubes of saline solution. Unless motion was seen, larvae that were uncoiled or showed definite degenerative changes were arbitrarily assumed to be dead. The results of this experiment are presented in Text-Figure 1 and show that

merthiolate when used as a sterilizing agent in this manner has a definite toxic effect which first becomes apparent 48 hours after the larvae are exposed. Quinanil has a similar but less marked effect. In similar experiments, a 5-minute exposure to a 1:500 dilution of sodium hypochlorite solution U.S.P. proved to be more toxic than merthiolate.

In an effort to avoid the toxic effect of chemical sterilizing agents an attempt was made to free the larvae of contaminating bacteria mechanically. By using the precautions outlined above in the section on isolation of larvae, and then by washing them for 5 minutes in each of six tubes containing 25 cc. of normal saline solution, a nematode suspension was obtained that was bacteriologically sterile when cultured aerobically and anaerobically, and which proved to be satisfactory as an inoculum for roller tube tissue cultures.

Attempts to Obtain Development in Roller Tube Tissue Cultures

Basic Technic. The basic technic employed was similar to that used recently for the culture of vaccinia virus (Feller, Enders and Weller, 1940). Reference should be made to this paper for details of the method. In brief, the cultures were prepared by planting fragments of minced 8- to 10-day-old chick embryo tissue in a chicken plasma clot distributed evenly over the wall of a 20 by 150 mm. pyrex test tube. Nutrient fluid consisting of 1.6 cc. of a mixture composed of Simms' solution, 7 parts, chicken embryonic extract, 2 parts, and chicken serum, 1 part, was added and the tube was then sealed with a one-holed rubber stopper fitted with a short piece of pyrex tubing, which in turn was closed with a rubber vaccine bottle cap. The cultures were placed horizontally in a rotating device which revolved 8 to 10 times every hour and were kept in an incubator at 37° C. Each day, after observations had been made, the nutrient fluid was removed through the small pyrex tube by means of a Pasteur pipette and 20 cc. of air which had been drawn through sterile cotton with a syringe was then introduced. Fresh nutrient fluid was added, the tube was sealed and returned to the incubator.

From 50 to 300 sterile larvae were introduced into each roller tube from 6 to 24 hours after the culture was assembled, with the shorter period of time giving the better results. Observations on the cultures were made with a low-power objective. Each time nutrient fluid was removed, the relatively few nemas suspended in the fluid were studied alive and then were fixed in a warm 4 per cent solution of formaldehyde. For the purposes of photomicrography, a few cultures were set up using roller bottles as described by Shaw, Kingsland and Brues (1940). These proved to be very satisfactory.

RESULTS OBTAINED WITH THE BASIC TECHNIC

Using the technic described above, partial development of the trichinella larvae was obtained. Although the nemas showed a decrease rather than an increase in size, and progressively died off, a small percentage molted twice and developed to the point of sexual differentiation.

Nineteen cultures were set up at various times using the basic technic. While there was some individual variation in the tubes, in general the results can be summarized as follows. Within 30 minutes after introduction of the larvae into the cultures, they showed vigorous activity, coiling and uncoiling rapidly, and then moving with a serpentine motion through the tissue. As migration began, the anterior tip vibrated rapidly, giving the impression that the nema was feeding. Although larvae molted for the first time as early as 16 hours after inoculation, the first ecdysis usually occurred between 24 and 36 hours after the culture was set up. Prior to molting there was a decrease in length, with retraction of the larva away from the old cuticular sheath both posteriorly and anteriorly. Coincidentally, a decrease in motility occurred, with movement being limited to a "to and fro" motion within the sheath and a fine vibratory motion of the anterior end. Molting was a slow process; one nema, which when first observed was half way out of its sheath, required 30 minutes to complete the procedure, which was accomplished by a slow backward and forward movement, accompanied by a lashing movement of the anterior free portion (Fig. 1). Upon completion of the molting process, the larvae were again extremely active and appeared to be feeding among the growing cells. The newly-escaped "second stage" larvae could be distinguished from those that had not molted by being shorter and thicker. They showed no sexual differentiation (Fig. 3).

From 10 to 20 per cent of the total number of larvae completed the first molt. Others failed to complete the first molt but began to show retraction in preparation for a second molt while still within the first sheath (Fig. 2). While many of the larvae that had molted once continued to develop and to show changes that were interpreted as being in preparation for further molts, only twice was the second molt observed; this occurred 48 hours after inoculation. These nemas showed a further decrease in size and the cast-off cuticula was smaller and more delicate than that shed during the first ecdysis. No sexual differentiation could be seen in this stage.

Further development was observed in larvae that failed to molt completely, but instead carried out an "incomplete molt" so that one sheath lay within the other. Retraction from a third cuticular sheath was first

seen at 38 hours and occurred frequently by the 50th hour of cultivation. Larvae that had retracted from the third sheath showed sexual differentiation with development of the vulva, ovary and uterus in the female and the appearance of anal papillae in the male (Figs. 4 and 8). By the 65th hour of cultivation such larvae had begun to show degenerative changes with beginning loss of internal structure; however, coincidentally there was a retraction from a fourth sheath (Fig. 6). In the male, the fourth sheath showed posteriorly a "cast" of the anal papillae (Figs. 5 and 7).

Only a small proportion of the larvae in each tube showed the developmental changes described above. Usually 80 per cent were alive at 24 hours, and about 60 per cent at 48 hours. By the end of the third day, degenerative changes began to appear and development ceased during the fourth or fifth day after inoculation, although a few degenerating larvae remained alive for as long as 9 days. During the period of development there was a decrease rather than an increase in size. Fifty larvae, killed in a warm 4 per cent solution of formaldehyde immediately after digestion, averaged $899\ \mu$ in length (extremes 805 to $1220\ \mu$). Ten males which had molted once, lying in two cuticular sheaths, and which showed well developed anal papillae, were killed in a warm 4 per cent solution of formaldehyde after 54 hours of incubation; they averaged $806\ \mu$ in length (extremes 670 to $950\ \mu$). Ten comparable females, each showing a well developed vulva, averaged $814\ \mu$ in length (extremes 700 to $925\ \mu$). The presence of the nemas in the cultures did not affect tissue growth. As in the virus experiments, the relatively large amounts of tissue used grew rapidly, with an accompanying fall in the pH from an initial value of about 7.8 to 7.0 or below in 24 hours.

Attempts to Obtain Further Development by Modification of the Basic Technic

Numerous experiments were carried out in an attempt to improve the technic described above. In each experiment one control roller tube was set up using the basic technic. Of the many combinations tried, not one proved to be more satisfactory than did the technic outlined above.

An attempt was made to determine if any of the components of the nutrient media had a deleterious effect upon the larvae. Sterile larvae were placed in Wassermann tubes containing 10 per cent chicken serum in normal saline, 20 per cent embryonic extract in normal saline, Simms' solution, and normal saline solution. After incubation for 72 hours at 37°C. , it was found that there was no significant difference between

the number of viable larvae in the control saline tube and in the embryonic extract and Simms' solution tubes; however, in the 10 per cent chicken serum tube, there were only one-fourth as many viable larvae. Therefore, roller tubes were set up using a nutrient fluid composed of two parts of embryonic extract and eight parts of Simms' solution; both larval development and tissue growth were poorer than in the control tube. Roller tubes were then set up using a nutrient fluid in which sheep serum was substituted for the chicken serum; excellent tissue growth but poor development of the nemas resulted. Mammalian embryonic tissue was substituted for the chick tissue by setting up roller tubes using rat embryos of approximately 18 days' gestation; this modification resulted in no significant change in the amount of development. Similar findings were obtained in an experiment planned to determine the effect of using various types of tissue in which 12-day-old chick embryos were dissected and separate roller tubes planted with liver tissue, intestinal tissue, and brain tissue. The possibility that the presence of an abrasive substance might assist in molting was studied by distributing sterile sand throughout the plasma coagulum in one set of tubes; no change was noted in the development of the larvae.

The behavior of the nemas suggested that some essential growth factor or factors might be lacking. A yeast extract was made using the method of Ferguson (1940); this was added to the nutrient fluid in various concentrations up to 10 per cent, either alone or in conjunction with added liver extracts. Liver extracts, which were used in concentrations up to 5 per cent, were prepared from Eli Lilly extract no. 343 by the method of Glaser and Coria (1933), and also from a crude aqueous liver concentrate* (1 cc. of concentrate was the equivalent of 0.03 lbs. of liver). The latter was employed by making a 1:1250 dilution in normal saline and sterilizing the solution by passage through a Seitz filter. Other cultures received nutrient fluid which, in addition to the usual components, contained 1 μ g. of ascorbic acid and 4 μ g. of glutathione per cc.; another set was given fluid containing 5 μ g. of thiamin hydrochloride and 1 μ g. of riboflavin per cc. In one group of tissue cultures the effect of adding split protein products was tried; to the standard nutrient media, an equal amount of 5 per cent aqueous bacto-tryptose, bacto-tryptone, or bacto-proteose peptone no. 3 † was added. In another set, 2.9 mg. of casein hydrolysate* was added to each 10 cc. of nutrient medium. All of the fluid media listed above gave fair to good tissue growth but did not significantly affect the development of the nemas. The addition of fresh human bile in concentrations of from 0.05 to 0.5 per cent and of sodium thioglycolate in concentra-

* Supplied through the courtesy of Lederle Laboratories, Inc., New York, N. Y.

† Produced by Difco Laboratories, Inc., Detroit, Michigan.

tions of from 0.001 to 0.005 per cent had a definite toxic effect upon the larvae.

DISCUSSION

Although numerous modifications of the basic technic failed to provide an environment that permitted complete development of trichinella larvae, it would appear from the results of the present study that the roller tube technic deserves further investigation, and may with only slight modifications prove to be a useful tool in the study of the helminthic parasites.

While no detailed morphological studies were made, the present findings are of interest in view of the uncertainty that exists regarding the number of molts that trichinella larvae undergo while developing in the intestine. Kreis (1937) recovered the developing larvae from infected rats at regular intervals and concluded that the female passed through four molts and the male through three molts. He noted no sexual differentiation until after the second molt, which occurred between the 12th and 16th hours of intestinal life. In the present study, sexual differentiation was first seen coincidentally with retraction from the third sheath. While the cultural results agree with those of Kreis as to the total number of molts in the female, in the present study males also were seen which had molted once and in addition were enclosed in three cuticular sheaths. This finding suggests that the male has four molts, although it is possible that one or more of the cuticular sheaths represent a response of the larva to the abnormal environment or else is a degenerative change. Chandler, Alicata and Chitwood (1941) felt that Kreis' evidence was not convincing and stated that "according to recent investigations one molt was obtained after ingestion and the cuticle of the resultant nema passed uninterrupted over the vulva, indicating that at least one more molt would be necessary before maturity." Inasmuch as in the present study the vulva was not seen until about the time of the third molt, this statement also conflicts with the observations reported above.

SUMMARY

In roller tube tissue cultures trichinella larvae developed to the stage of sexual differentiation. While the nemas decreased rather than increased in size, a few larvae completed two molts. A larger number of larvae completed one molt, and then progressed through three additional "incomplete molts," so that nemas were seen lying within three distinct cuticular sheaths. Anal papillae in the male and the vulva in the female became prominent after the third "incomplete molt." These findings suggest, but do not prove, that both male and female trichinella larvae molt four times during the intestinal phase of their life cycle.

Agents previously used for sterilizing nemas were found to have a toxic effect on trichinella larvae and therefore a simple washing technic was developed which yielded bacteriologically sterile larvae that were suitable for introduction into tissue cultures.

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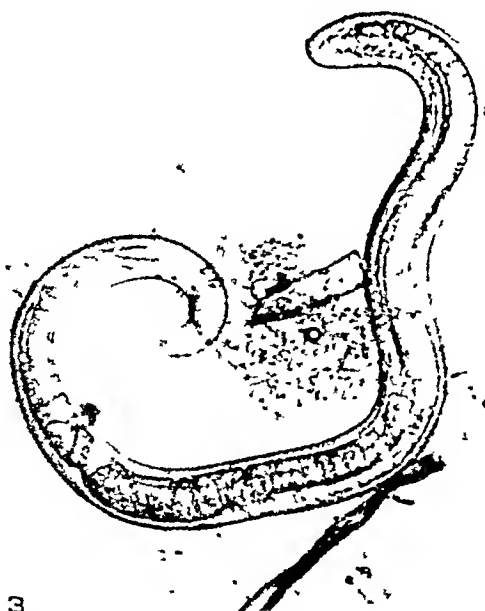
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 55

- FIG. 1. Live larva (*Trichinella spiralis*) molting for the first time. The free portion was moving rapidly. Photographed in roller bottle tissue culture after 26 hours' incubation.
- FIG. 2. Live larva in roller bottle tissue culture after 38 hours' incubation showing an "incomplete" first molt and retraction from a second cuticular sheath in preparation for a second molt.
- FIG. 3. Live larva in roller bottle tissue culture shortly after completing the first molt. Taken after 28 hours' incubation. The anterior tip was vibrating rapidly.
- FIG. 4. View of vulvar region of female nema shown in Figure 8. Heat-killed after 48 hours' incubation.

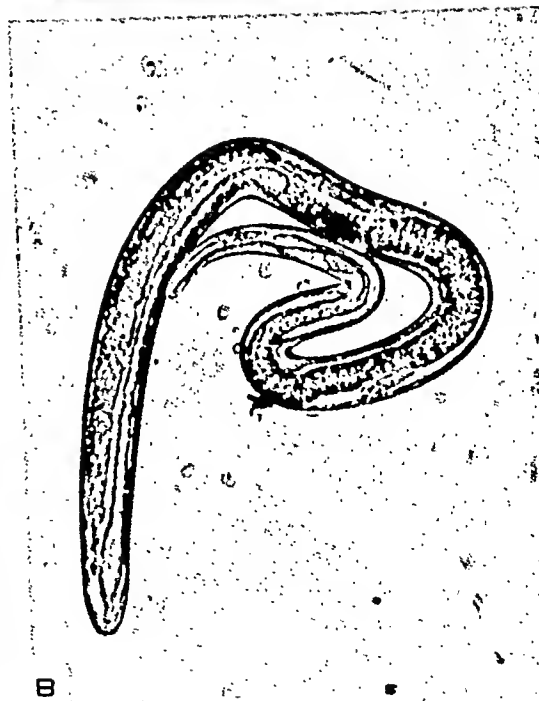
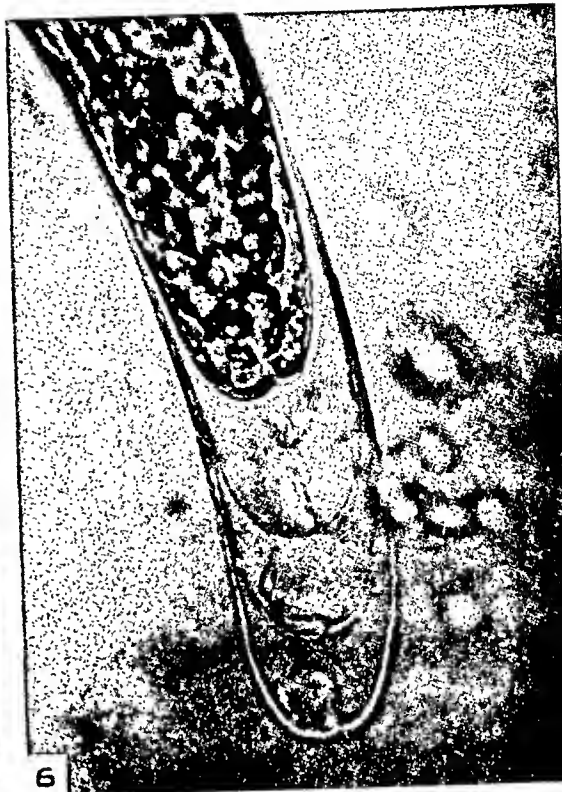
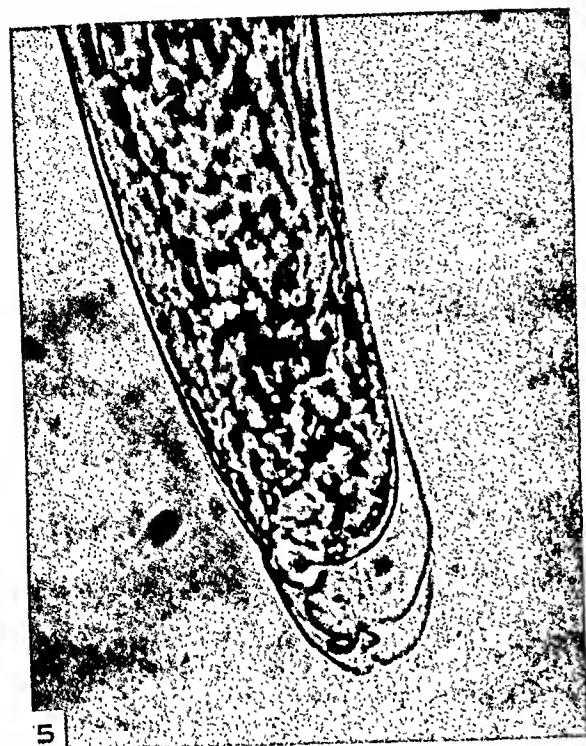


Weller

Development of *Trichinella spiralis*

PLATE 56

- FIG. 5. Posterior end of male with well developed anal papillae. This nema probably had molted twice, and lies within a smooth third cuticular sheath, and a fourth sheath that shows a cast of the anal papillae. Heat-killed after 58 hours' incubation.
- FIG. 6. Posterior end of female that had molted once, showing retraction from three additional cuticular sheaths. Heat-killed after 70 hours' incubation.
- FIG. 7. Low-power view of male shown in Figure 5. Shows maximum development obtained. Heat-killed after 58 hours' incubation.
- FIG. 8. Low-power view of female pictured in Figure 4, showing maximum development obtained. This nema had molted once, and was lying within two additional cuticular sheaths. Heat-killed after 48 hours' incubation.



Weller

Development of *Trichinella spiralis*

EFFECTS OF INFRARED IRRADIATION ON THE TISSUES OF THE RABBIT*

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(From the Department of Pathology, University of Tennessee, Memphis, Tenn.)

Many data have accumulated on the effects of roentgen irradiation on both normal and pathological tissues, yet the exact method by which the effects of these rays are produced is not thoroughly understood. Ellinger¹ has recently compiled much of the literature on the mode of action of irradiation on cells. Few studies, however, have been made on the pathological changes that follow ultraviolet irradiation and still fewer on the effects produced by infrared.

All types of irradiation may produce injury to cells, according to the photochemical theory. Various factors, of course, influence these changes. The type of cell, the degree of absorption and the intensity of the irradiation are important. Roentgen rays are very penetrating and carry large amounts of energy. Ultraviolet radiations are characterized by their less penetrating powers; however, they initiate chemical changes in a large number of biologically important substances.¹ The ultimate effects of infrared irradiation may be the same as roentgen and ultraviolet irradiation.

Recently experimental studies on the effect of roentgen irradiation on capillary permeability and inflammation, and of ultraviolet irradiation on the localization and concentration of antibodies in the skin of the rabbit have been made.^{2,3} Infrared irradiation also was used to determine its effect on these processes. Marked pathological changes occurred in the skin and viscera of the rabbits treated with infrared rays. This paper is a report of these effects.

METHODS AND MATERIALS

Adult rabbits were used. The hair was shaved from the sides and abdomen 24 hours or longer before the experiments were begun. Infrared irradiation was obtained from a lamp † placed 10 inches above the area of skin to be irradiated. The electric current used in this lamp was 110 volt, 60 cycle A.C.; 220 watts. The energy distribution graph with the spectral distribution of the output of this lamp is shown in Text-Figure 1.

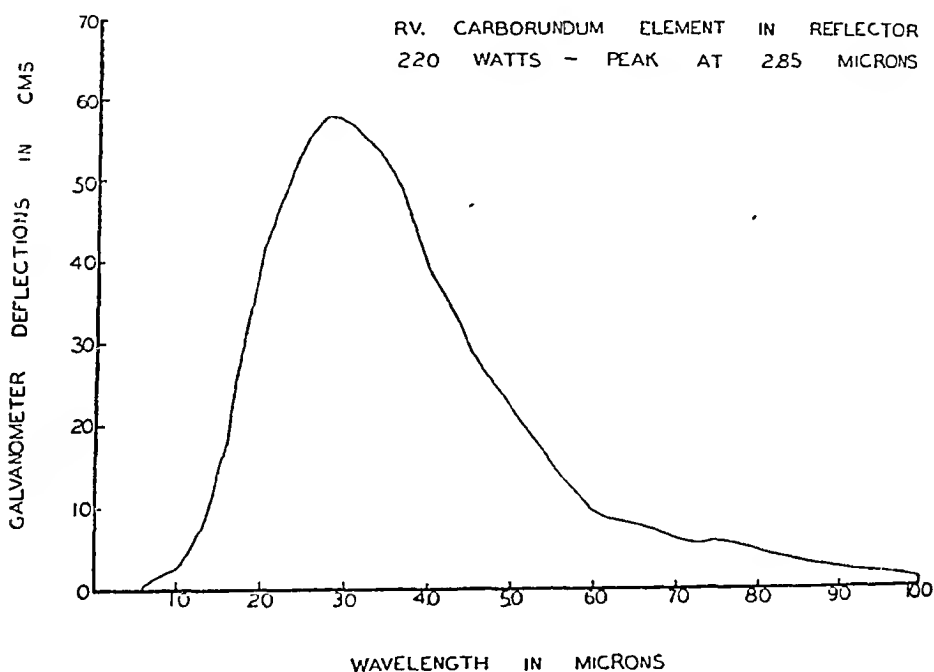
* Aided by grants from the International Cancer Foundation and the University of Tennessee.

Received for publication, October 12, 1942.

† The lamp is a Zoalite, type Z-70, supplied by the Burdick Corporation, Milton, Wisconsin. The data for Text-Figure 1 were also furnished to us by that company. We acknowledge our appreciation for this co-operation.

A cloth towel with a hole 3.5 cm. in diameter was placed over the shaved skin of some of the animals. Some of the rabbits were anesthetized with pentobarbital when the exposure was made for a long period. The length of the exposure of the skin of these animals to infrared varied from 1 to 5 minutes. The longer intervals were used to produce lesions in the viscera.

Ten cc. of a 0.2 per cent solution of trypan blue was injected intravenously immediately following irradiation. The rabbits were killed at intervals varying from immediately after injection to 144 hours. Autopsies were performed at once to determine the areas in which the



Text-Figure 1. The energy distribution graph with the spectral distribution of the output of the lamp used in these experiments.

trypan blue had localized and concentrated. Sections were removed and fixed immediately in a 4 per cent solution of formaldehyde. Paraffin sections were prepared and stained with hematoxylin and eosin.

EFFECT OF INFRARED IRRADIATION ON THE RABBIT AS OBSERVED BY THE INTRAVENOUS INJECTION OF TRYPAN BLUE

Infrared light, when applied to the skin of the rabbit, produced almost instantaneous blanching and then, very quickly, hyperemia. Edema subsequently developed. Trypan blue, when given intravenously immediately following the irradiation, localized and concentrated throughout this hyperemic area. When the amount of irradiation was markedly

increased, the tissue directly beneath the arc of the lamp remained pale yellow in color, and a zone at the periphery became hyperemic and edematous. Trypan blue was localized and concentrated only in the zone about the periphery of the pale yellow area. Histological studies made subsequently indicated that the cells were completely destroyed in the center of this area and only injured about the periphery. This observation indicates that trypan blue will be localized and concentrated only in areas where the cells are injured and not completely destroyed.

When infrared irradiation was applied to the skin for a period of 5 minutes and trypan blue was given intravenously immediately thereafter, the dye localized and concentrated in the abdominal muscle and in the portion of intestine just beneath the irradiated area of skin. It was apparent that infrared rays, as used in these experiments, penetrated the abdominal wall of the rabbit and produced changes in the permeability of the cells in the intestines similar to the changes that occurred in the skin.

Histological Lesions Observed in the Rabbit Following Infrared Irradiation

Skin. The epithelial cells showed injury of various types. These were influenced by the length of the exposure. The subcutaneous tissue became edematous and its cells pyknotic. Many cells resembling plasma cells infiltrated the corium. Polymorphonuclear leukocytes sometimes infiltrated the corium immediately beneath the squamous epithelium. The endothelial cells of the small blood vessels became swollen and pyknotic. Thrombi in the smaller blood vessels were rarely seen except in those rabbits given very large amounts of the irradiation.

Muscle. The muscle in the abdominal wall appeared to be very susceptible to the effects of infrared irradiation. Groups of muscle cells frequently became swollen and lost their striations within a period of 30 minutes following irradiation. Many of these muscle cells were necrotic and were fragmented within an hour. Polymorphonuclear leukocytes frequently infiltrated the tissue about these degenerating muscular fibers.

Gastrointestinal Tract. Ulcers were found to occur in any portion of the intestinal tract following exposure of the abdomen to infrared irradiation for a period of 3 to 5 minutes. It was necessary to concentrate the irradiation in the area of the stomach to produce ulcerative lesions in that organ. Likewise, it was necessary to place the light over the skin in the area of the small intestine and colon to produce lesions there. The earliest lesion observed macroscopically was hemorrhage

into the mucosa. Apparently, lesions such as this subsequently developed into ulcers. The size and number of ulcers varied in different rabbits. Lesions in the stomach and the cecum are shown in Figures 1 and 2.

The epithelial cells apparently were very susceptible to the effect of infrared irradiation. These cells showed all types of degenerative changes. Karyorrhexis was very marked in some of the lesions. Figure 3 shows the accumulation of degenerated epithelial cells in the lumen of the glands in the mucosa of the colon. Similar changes occurred in the epithelial cells in the stomach and the small intestine. The entire wall of the colon became edematous in the area of the ulcers. Polymorphonuclear leukocytes infiltrated the portion of the intestine injured by irradiation.

The cells in the lymphoid tissue in the intestinal tract appeared to be much more resistant to the action of infrared irradiation than the columnar epithelial cells. The epithelial cells covering a Peyer's patch and also the cells lining the glands in the area were severely injured by infrared irradiation while the lymphocytes did not show any specific degenerative changes (Figs. 4 and 5).

Liver. Focal yellowish red areas were present in the livers of some of the rabbits. This lesion extended for only a short distance into the hepatic tissue. Always the lesion occurred at the periphery of the organ. Histologically the hepatic cells in these areas showed all stages of injury from cloudy swelling and vacuolization of the cytoplasm to karyolysis. In some of the animals, polymorphonuclear leukocytes infiltrated the area of degeneration. There was always a sharp line of demarcation between the injured and the normal cells (Fig. 13).

Spleen. Lesions seldom occurred in the spleen of this group of rabbits. Figure 10 shows several focal areas of necrosis. These were definitely localized and appeared as infarcts. Histologically the splenic tissue showed degenerative changes, and polymorphonuclear leukocytes were found in a pyogenic membrane about the periphery of the area. It is important to observe (in Fig. 12) a splenic vessel in this area, in which the lumen is occluded by a pink-staining, fibrinlike material. The wall is necrotic. The presence of this vascular lesion suggests that the degeneration in the surrounding splenic tissue results from it rather than from the direct effects of infrared irradiation on the splenic tissue, but that the lesion in the vessel was itself due to irradiation.

Kidneys and Adrenals. No pathological changes were observed in the kidneys and adrenals. This may be attributed to an insufficient quantity of infrared irradiation reaching these tissues.

Lungs. A few of the rabbits showed focal hemorrhagic areas and some edema in the lungs following irradiation of the abdomen. A more

extensive lesion occurred when the light was applied to the skin directly over the chest wall.

Heart. No definite pathological lesions were observed.

Femoral Bone Marrow. A posterior extremity of each of five rabbits was shaven. Infrared irradiation was applied to the thigh for a period of 5 minutes. Six hours later the animals were killed and the femoral bone marrow removed. Marrow was removed from a corresponding portion of the opposite leg. There were no pathological changes observed in the sections of the marrow from either leg.

Cranial Bone Marrow. A portion of the skull in the area treated with infrared irradiation was removed from some of the rabbits. The marrow here was hemorrhagic and the cells showed marked degenerative changes. The skull in the rabbit is relatively thin as compared with the shaft of the femur. It is likely that the femur is too dense for the penetration of these infrared rays.

Brain. Lesions were present in the brain of each rabbit in which infrared irradiation was used on the head. The light was applied to the area between the ears. The underlying portion of the brain was the only portion to be affected (Fig. 6). Petechiae occurred along each side of the median line. The brain cells in this area were pyknotic and showed karyorrhexis. Clear spaces were present around many of these cells. These areas were thought to represent areas of edema. The tissue was necrotic in some of the animals (Figs. 7 and 8). A leukocytic zone was present in the brain when an interval of several hours elapsed between the application of the light and the taking of the section (Fig. 9). The pathological changes in the brain usually extended for only a short distance into the cortex; however, in a few of the brains the lesions extended almost to the lateral ventricle.

Eye. The soft tissues about the eyes of rabbits receiving infrared irradiation over the skull were edematous and hemorrhagic. The epithelial cells of the cornea were frequently infiltrated with polymorphonuclear leukocytes. The cells in the retina also showed degenerative changes and leukocytes infiltrated the area. The lens frequently appeared opaque in the rabbits that lived for several hours following application of the light.

DISCUSSION

The results of these studies indicate that a change may occur in the tissues immediately following infrared irradiation. The localization and the concentration of trypan blue following an intravenous injection indicate that these tissues vary from the normal. The subsequent histological lesions are a confirmation of this injury.

It is widely held that a histamine-like substance is liberated from the

tissues following irradiation and that the subsequent changes result from the presence of this substance.⁴ It has been suggested, however, that the local changes which occur in inflammation may result from the direct action of the injurious agent on the cells rather than from the effect of a substance liberated from injured tissue.⁵ The direct effect of these rays on endothelial cells in the blood vessels of the corium apparently is sufficient to produce changes in these cells that result in an increased permeability. Borak⁶ has recently expressed the opinion that x-rays act on the large protein molecules to break them down to smaller ones, hence increasing the osmotic pressure and causing the flow of liquid into the cells, often reaching the point of cellular disintegration. This phenomenon occurs in both the endothelial cells and the extravascular tissue, according to Borak.

The presence of degenerative lesions in the intestinal mucosa indicates that infrared rays penetrated the abdominal wall.

It is impossible to compare the results of the effects of infrared irradiation in this study with the results of others with roentgen rays; however, it is of interest to note that Ellinger,¹ in 1941, stated that the adult brain and nervous system are relatively radioresistant; muscle is one of the least radiosensitive tissues; the kidneys are not particularly radiosensitive; the liver is decidedly radiosensitive; the intestines also are radiosensitive. It appears from the present study that the skin, muscle, gastrointestinal tract, liver and brain are very susceptible to the effects of infrared irradiation. The apparent resistance of the heart and kidney to infrared irradiation may be explained by the decreased amounts of irradiation reaching these organs.

Warren and Whipple⁷ observed that the epithelial cells in the mucosa of the gastrointestinal tract were more susceptible to the effects of roentgen irradiation than the lymphoid cells in the underlying Peyer's patches. It is of interest to observe a similar relationship between the susceptibility of the epithelial and the lymphoid cells following the application of infrared irradiation to the intestines of the rabbit.

Degenerative changes in the cells of the bone marrow in the skull and the absence of lesions in the same cells in the femoral bone marrow would suggest that an insufficient amount of radiation is absorbed by the latter cells. Hofmann⁸ and Heald⁹ have shown that infrared radiation is transmitted through the hand and forearm of man. These observations of Hofmann and Heald, however, indicate that the rays penetrated only the soft tissues and not the osseous tissue. It would appear likely from these histological studies that infrared irradiation may produce cellular degeneration in any tissue if the concentration of radiation reaching the tissue is sufficient.

A knowledge of the potential effects of infrared irradiation is important in view of its increased clinical use. In regard to its use, Beaumont¹⁰ has said: "It is evident to all those who have extensive opportunities for the close clinical observations of a large number of cases that there is some factor other than heat responsible in some measure for the results obtained."

SUMMARY

The pathological changes that occur in the rabbit following the application of infrared irradiation to the skin are described. These are characterized by extensive necrosis and ulceration. Lesions are present in the skin, abdominal muscles, stomach, intestine, spleen, liver, lungs, brain, eyes and bone marrow.

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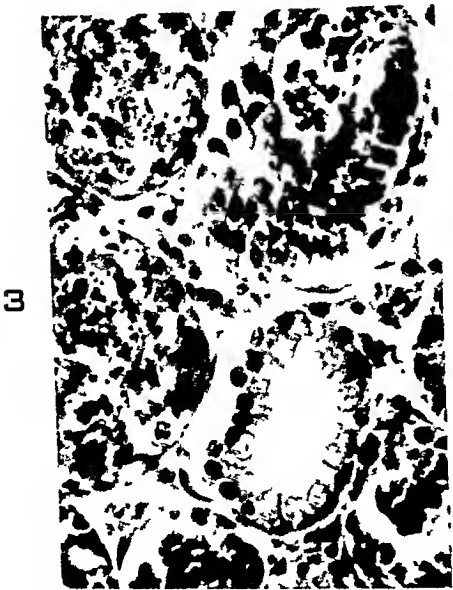
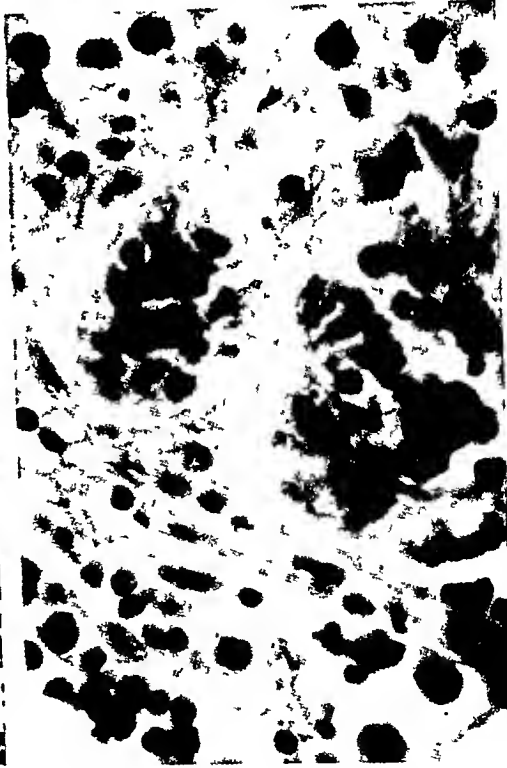
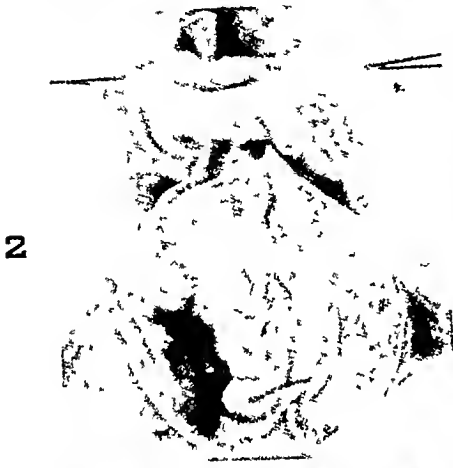
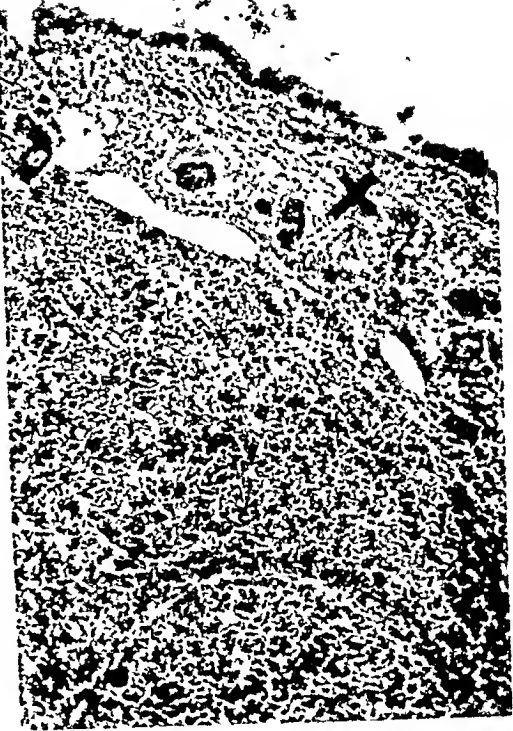
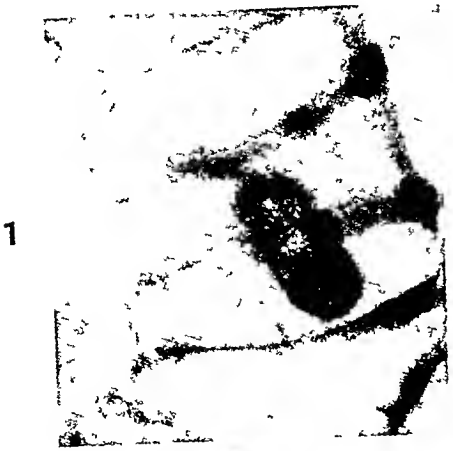
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 57

- FIG. 1. Rabbit 1239. Acute ulcer in the cecum. Lesions similar to this occur also in the colon and small intestines. Usually they are multiple. Infrared irradiation was applied to the abdomen for 5 minutes at a distance of 10 inches from the skin. The cecum was removed 5 hours following irradiation.
- FIG. 2. Rabbit 1234. Ulcers in the mucosa of the stomach. They are similar to those in the cecum. This rabbit was given the same irradiation as rabbit 1239, the cecum of which is shown in Figure 1, and was killed 24 hours later.
- FIG. 3. Rabbit 1101. The epithelial cells lining the glands in the mucosa of the colon show extensive degenerative changes. The lumina of the individual glands are usually filled with cellular debris. This rabbit was irradiated for 4 minutes at a distance of 12 inches. Section removed 30 minutes following the irradiation. $\times 100$.
- FIGS. 4 and 5. Rabbit 1137. The epithelial cells covering a Peyer's patch and those lining the glands show an extensive degenerative change. No changes are observed in the lymphocytes. The section shown in Figure 5 was removed immediately below X in Figure 4. The skin was irradiated for 4 minutes at a distance of 12 inches. Killed 15 minutes following irradiation. Figure 4, $\times 36$; Figure 5, $\times 375$.



Rigdon, Ewing and Tate

Effects of Infrared Irradiation

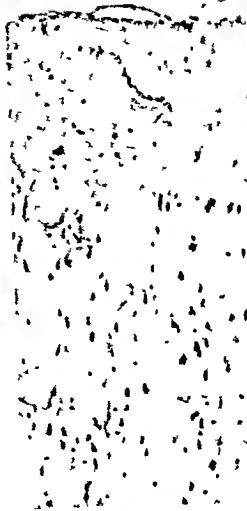
PLATE 58

- FIG. 6. Rabbit 1290. Hemorrhages and necroses occur in the area of the brain immediately beneath the region irradiated. Infrared irradiation applied for 5 minutes at a distance of 10 inches. The rabbit was killed 24 hours following irradiation.
- FIGS. 7 and 8. Rabbit 1290. A portion of the cerebral cortex unaffected by the infrared irradiation and a similar area of the cortex showing degeneration of the cells and small hemorrhages 24 hours following irradiation. Infrared irradiation was applied for 5 minutes at a distance of 10 inches. Killed 24 hours following irradiation. $\times 100$.
- FIG. 9. Rabbit 1291. The periphery of the areas of necrosis in the brain frequently has a zone of leukocytes about it. The skin over the skull was irradiated for 5 minutes at a distance of 10 inches. $\times 100$.
- FIG. 10. Rabbit 1234. Focal areas of necrosis may occur in the spleen. Infrared irradiation was applied to the skin over the splenic area at a distance of 10 inches for 5 minutes. Killed 24 hours later.
- FIG. 11. Rabbit 1234. The lungs are frequently hemorrhagic and congested. Irradiated for 5 minutes at a distance of 10 inches. Killed 24 hours later.
- FIG. 12. Rabbit 1234, the same animal as used for Figure 10. In the center of a focal area of necrosis there is a thrombosed artery. It is probable that the necrosis resulted from this vascular occlusion, and that the irradiation produced the vascular lesion. $\times 36$.
- FIG. 13. Rabbit 1242. A focal area of necrosis near the periphery of the liver after the infrared light was placed over this organ. The hepatic cells are severely damaged. Irradiated for 5 minutes at a distance of 10 inches. Killed 24 hours later. $\times 100$.

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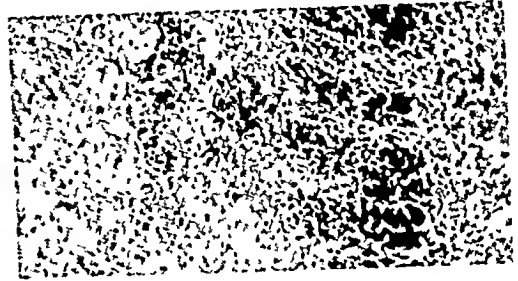
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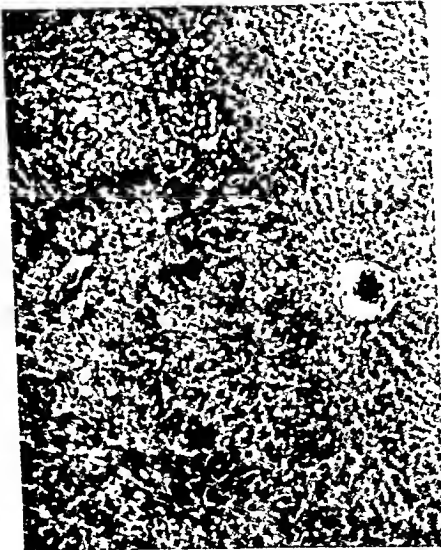
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Rigdon, Ewing and Tate

Effects of Infrared Irradiation



REPORT OF THE MEETING OF THE COUNCIL
THE AMERICAN ASSOCIATION OF
PATHOLOGISTS AND BACTERIOLOGISTS

CLEVELAND
APRIL TENTH, 1943

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Report of the Meeting of the Council
Held at Cleveland, Ohio, April 10, 1943

Present. President CANNON, Doctors FORBUS, GOODPASTURE, HAYTHORN, KARSNER, SOULE, WARREN and WELLER.

The following were elected to membership in the Association:

ERNEST E. AEGERTER	WEBB HAYMAKER
HILDEGARDE ARNOLD	PETER A. HERBUT
OSCAR AUERBACH	AMBROSE J. HERTZOG
ALICE I. BERNHEIM	ROBERT C. HORN, JR.
ALBERT F. BROWN	ROBERT S. JASON
CHESTER R. BROWN	HERMAN JOSEPHY
FREDERICK I. DESSAU	HERMANN LISCO
MARTIN L. DREYFUSS	JOSEPH M. LUBITZ
CHARLES E. DUNLAP	RICHARD M. MULLIGAN
HENRY W. EDMONDS	CHARLES R. REIN
HUGH A. EDMONDSON	RAYMOND H. RIGDON
GEORGE L. FITE	WALTER H. SHELDON
A. JAMES FRENCH	DAVID M. SPAIN
ERVING F. GEEVER	SIEGFRIED TANNHAUSER
ANGELO M. GNASSI	RALPH M. THOMPSON
THOMAS A. GONZALES	PHILIP WASSERMAN
WILLIAM H. HARRIS, JR.	FREDERICK R. WEEDON
MARK C. WHEELOCK	

The deaths of the following members were recorded with deep regret:

L. K. BALDAUF
E. A. BAUMGARTNER
WADE H. BROWN

Cancellation of Annual Meeting for 1943. In view of the various problems connected with the war, the Council voted by mail to cancel the annual meeting of 1943.

Since no meeting was held, it was impossible to elect new officers for the ensuing year. The Secretary read Article II of the Constitution as follows: "The business of the Association, including the election of members, shall be conducted by a Council, which shall nominate an-

nually, to be elected by the Society, a President, a Vice President, a Secretary and a Treasurer, to perform the duties usually devolving upon such officers. The same person shall not serve as President more than one year consecutively."

There was extensive discussion of the problem created by the cancellation of the annual meeting. As a result, it was voted that the Council, empowered to conduct the business of the Association, interpret the Constitution to the effect that, as ordinarily provided in more elaborate documents of this sort, the officers shall retain their positions until such time as their successors shall qualify; that this statement be published in the report of the Council in the *American Journal of Pathology*, and that members of the Association who dissent are invited to communicate with the Secretary.

Meeting Place for 1944. Dr. Cannon reported that the invitation of the University of Chicago holds for the meeting in 1944. It was voted to accept gratefully this invitation, provided conditions are such that a meeting can be held in 1944.

Symposium for 1944. It was voted that at the next meeting of this Association, whether in 1944 or subsequently, the topic for the symposium be "Infectious Granulomas, Exclusive of Tuberculosis and Syphilis," and that Dr. Wiley D. Forbus be requested to act as referee. Dr. Forbus accepted this assignment.

Funds of the Congress of American Physicians and Surgeons. The Secretary reported that the funds which had accumulated in the treasury of the Congress of American Physicians and Surgeons had, as a result of the abandonment of the Congress, been turned over to the American Red Cross Society.

Change in Fiscal Year of Association. It was voted to change the fiscal year of the Association to correspond to the calendar year and to the volume year of the *American Journal of Pathology*. Bills to members will be mailed early in January of each year. If payment is not made by March 1, a second notice, indicating delinquency, will be mailed and if payment is not made by April 1, the names of those still delinquent will be removed from the mailing list of the Journal. New members will be billed immediately after election and upon payment they will receive the Journal beginning with the first number of the current year.

HOWARD T. KARSNER, *Secretary*

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NUMBER 4

SCLEROSING HEMANGIOMAS

THEIR RELATIONSHIP TO DERMATOFIBROMA, HISTIOCYTOMA, XANTHOMA AND TO CERTAIN PIGMENTED LESIONS OF THE SKIN *

ROBERT E. GROSS, M.D., and S. BURT WOLBACH, M.D.

(From the Departments of Pathology of the Harvard Medical School, the Peter Bent Brigham Hospital, and the Children's Hospital, Boston, Mass.)

The histologic classification of tumors is often decided by comparing average fields in different lesions and grouping together those which match most closely. Changes of secondary character, however, may make tumors of the same origin appear to be different on superficial examination. As a result, tumors originating from identical cells have come to be known by several different names. If the classification is based on the cells of origin, and the relation of secondary changes to the final tissue pattern is recognized, then one can establish groups of tumors which are similar in origin and behavior.

Few, if any, classes of tumors have become more confused by the practice of matching average pictures than have the hemangiomas. Pathologists and clinicians have long recognized as hemangiomas the red or bluish cutaneous lesions which histologically are composed of capillaries or cavernous blood spaces. They have also observed some blood vessel tumors of the skin to undergo regression or even to disappear without treatment. Regression may be precipitated by local injury, by surface ulceration, or by superimposed infection. These changes are well known; an extensive study of them has been published by Lister.¹

It is not well known that a process of sclerosis without detectable antecedents frequently occurs in hemangiomas, a process which results in striking changes in the gross and microscopic appearance of these lesions. The overgrowth of fibrous tissue in hemangiomas leads, by a definite series of histologic sequences, to the accumulation of fat and hemosiderin. These changes take place in varying degrees, one predominating in one specimen, another in another. The process of sclerosis may, therefore, lead to a number of different end-results.

* Received for publication, December 10, 1942.

The tumors when removed are often in one or another of these end-stages, the steps in the progressive tissue sequences being obscured. When the lesions are deeply pigmented with hemosiderin, they have been confused clinically and even pathologically with melanomas (Figs. 1 and 10). When they are filled with fat, they are sometimes regarded as cutaneous xanthomas (Figs. 3, 5 and 11) or "giant cell xanthomas," if there are many giant cells in addition to the lipoid-filled cells. When the connective tissue overgrowth is prominent and accumulation of both fat and hemosiderin is slight, the lesions have been placed in the group of unencapsulated fibromas (Fig. 7). The terms *histiocytoma*, *dermatofibroma* and other names have been applied to one or another phase of sclerosing hemangiomas.

It seems, therefore, advisable to trace the process of sclerosis in hemangiomas through a series of the lesions. In this way, the possible variations may be defined more clearly and the essential similarity of lesions which appear very different may be established. The identification of these lesions as sclerosing hemangiomas is important because it shows that they are benign and localized; they are not cutaneous manifestations of a generalized disease. These microscopic changes were recognized and described briefly by one of us (S. B. W.)² in 1913; the present material is in accord with the views expressed in that paper.

MATERIAL FOR STUDY

All records and microscopic sections of hemangiomas from the files of the Departments of Pathology of the Peter Bent Brigham Hospital and the Children's Hospital were reviewed. The great majority of these hemangiomas were from surgical specimens; only a few had been removed at autopsy. Those which showed histologic pictures suitable for inclusion in the category of "sclerosing hemangioma" were selected for study. A total of 67 specimens was thus obtained, and from this material the following descriptions have been made. Paraffin sections in all instances were stained with either eosin and methylene blue, or hematoxylin and eosin. The majority were stained also with phosphotungstic acid hematoxylin and Mallory's aniline blue connective tissue stain. Many were studied with fat stains (scharlach R) applied to frozen sections of formaldehyde-fixed tissue and with iron stains (Berlin blue or Turnbull's blue) on paraffin sections. In some instances Foot's reticulum stain, Weigert's elastic tissue stain, and osmic acid staining for fat were used for demonstration of special features.

DISTRIBUTION AND GROSS APPEARANCE OF SCLEROSING HEMANGIOMAS

The 67 sclerosing hemangiomas from 66 patients were distributed as follows:

Upper extremity	15
Location on extremity not specified	4
Upper arm	1
Forearm	3
Wrist	1
Palm	1
Fingers	5
Lower extremity	29
Location on extremity not specified	4
Thigh	6
Around knee	5
Calf	2
Shin	6
Ankle	2
Foot	2
Toes	2
Face	5
Eyelid	2
Shoulder	3
Breast	3
Abdomen	2
Buttocks	3
Back	1
Scattered	1
Region not stated	3

When grouped according to age of the patients, the following results were obtained:

Age in years	Number of patients	Percentage of series
0-9	5	7
10-19	8	12
20-29	9	13
30-39	14	21
40-49	15	23
50-59	7	11
60-69	2	3
Age not stated	6	10

Eighty per cent of the patients were between the ages of 10 and 59 years; 44 per cent were between 30 and 49 years. The high incidence of sclerosing hemangiomas in the older decades is in distinct contrast to the relative frequency with which cutaneous (unchanged) hemangiomata are observed in childhood as compared with adult life. This provides some evidence that the sclerosing process requires years or even decades to be completed.

Cutaneous sclerosing hemangiomas are commonly limited to the skin and subcutaneous fat. It is rare for them to extend as deeply as the superficial fascia or the skeletal musculature. The records indicated considerable variety in the size, shape and color of the skin lesions. The smallest were but a few millimeters in diameter; others were as large as 4 or 5 cm. in cross dimensions (Fig. 1). Average examples were 8 to 12 mm. in diameter. Most of them were flat lesions, covered by skin which was somewhat smoothed out. At times, however, the overlying skin retained its normal dimples and fissures. The lesions usually lay in the corium and subcutaneous fat and did not project above the level of the surrounding epidermis. In only a few examples did they rise appreciably above the adjacent skin; one specimen was pedunculated. The color ranged from a gray or pinkish gray (when fibrosis was extensive) to a pale yellow (when lipoid deposits were heavy) or to tan-brown (when pigment accumulations were marked) (Fig. 1). Frequently the patient would volunteer the information that a "birthmark" or typical angioma had been present for many years but that subsequently it had gradually assumed its present appearance.

Cross sections of some examples showed little more than a thickening and increased stiffness of the corium; generally there was sufficient fibrosis to give the specimen a firm consistency. Usually the lesion could be clearly outlined with the naked eye, but there was no encapsulation and the pathologic tissue merged into surrounding normal structures (Fig. 6). If there was a large amount of fibrosis, the fresh tissue had a light gray or pearly appearance. If large quantities of lipoid were present, the cut surface was yellow, with the intensity directly related to the amount of accumulated fat. Similarly, deposits of pigments imparted varying shades of tan or brown to the cut surface (Fig. 2) depending upon the amount present.

HISTOLOGIC SEQUENCES IN SCLEROSING HEMANGIOMAS

In all hemangiomas there are two tissues—the blood vessels and a supporting connective tissue stroma. The process of sclerosis consists of an overgrowth of the stroma; in most instances the increase in connective tissue initiates a series of histologic sequences which might appear to be entirely unrelated.

The extent of connective tissue overgrowth is extremely variable. When a number of specimens are studied all gradations of sclerosis can be identified, from that which is but a slight increase over the usual stroma to that which represents almost complete obliteration of the

blood vessels by dense collagenous tissue (Fig. 8). Occasionally, the fibrosis is so marked that it is almost impossible to recognize the hemangiomatous origin of the lesion. Different areas in a single specimen may also show wide variations in the amount and character of connective tissue proliferation. Often, there is a well advanced sclerosis in the central portion of the specimen while the peripheral zones show actively proliferating angiomatous tissue little affected by the process of sclerosis. The intervacular connective tissue may be very cellular and composed of large, young fibroblasts or it may have a low cellularity and be composed of shrunken, mature, connective tissue cells with a large amount of collagen. In the latter cases, the collagen is often hyalinized.

While collagen may be extensively formed, elastic tissue is rarely found; when elastic fibers are seen, they are short, thin and widely separated, compared to those of normal corium. The fibers appear to be those of the corium present before the process of sclerosis was initiated. The elastic tissue does not seem to be newly formed in the connective tissue overgrowth.

The sclerosing process has a distinct effect on the vascular network in the hemangiomas. Concentrically arranged fibroblasts encircle and constrict individual capillaries. The gradual contraction of connective tissue compresses the blood vessels of the lesions so that their lumina are progressively reduced in size. Portions of the vessels are then cut off from the circulating blood stream, and obliteration of channels finally occurs. The progressive occlusion of the angiomatous channels is seldom accompanied by thrombosis. After complete closure of vessels has taken place, the cords of remaining endothelial cells are broken up and nests of them become pinched off in such manner that their relation to the rest of the blood vessel is lost.

The endothelial cells, thus isolated, at times coalesce to form giant cells (Fig. 9). Giant cells were found in 18 of the 67 specimens in the series. In some of the 18 tumors, the cells were few but occasionally they were so numerous as to dominate the histologic picture. In sclerosing capillary hemangiomas, cords of obliterated vessels give rise to giant cells. In those specimens where a more rapid growth is evident (hemangio-endothelioma), sheets of endothelial cells appear to differentiate directly, either into capillary blood vessels or into giant cells. Wolbach,² and later Kirch,³ described hemangio-endotheliomas of the skin which had become so altered that they possessed the histologic appearances of giant cell xanthomas; the present report is in entire agreement with the views expressed in these two papers.

The giant cells are of the "foreign body" type, and contain from two or three to as many as twenty or more nuclei (Fig. 9). Their cytoplasm takes a deep stain and is more basophilic than that of surrounding cells. It is often homogeneous. In some areas, however, the cytoplasm of the giant cells contains fat droplets, or hemosiderin, or both. These materials often accumulate in other cells of endothelial origin in sclerosing hemangiomas, as subsequently described.

One of the most striking results of the overgrowth of stroma in hemangiomas is the accumulation of hemosiderin (Fig. 4). Of the 67 specimens on which the present study is based, pigment deposits were marked in 7, moderate in 9, slight in 12, and absent in 39. While some of the pigment is free in the tissues, most of it is within the cytoplasm of endothelial cells. The evidence that the cells are of endothelial origin lies in part in their morphologic resemblance to the cells lining nearby blood vessels. Additional evidence is derived from the fact that hemosiderin is occasionally seen in endothelial cells lining still patent capillaries in these lesions (Fig. 10). The amount of pigment in some cases is so great as to impart a deep brown color to the lesions (Fig. 1). These deeply pigmented sclerosing hemangiomas are easily confused clinically with melanomas.

It is difficult to explain the most marked degrees of pigmentation by disintegration of entrapped red blood cells alone, especially in view of the fact that there is no definite correlation between degree of pigmentation and degree of sclerosis. In occasional specimens there is evidence of old or recent hemorrhage, and some of the pigment is possibly derived from this source. In the vast majority of cases, however, no hemorrhage is apparent and the source of the excess pigment remains unexplained.

The pigment is light golden brown, is doubly refractile and has all the appearances and staining reactions of hemosiderin. It is found in globules of irregular shape and size, in contrast to the rather small, rounded, and more uniform size of melanin granules (Smith⁴). It is stained an intense blue by the Berlin blue and the Turnbull's blue reactions (Fig. 4)—a fact which establishes the differentiation from melanin. This test can be made on Zenker-fixed material embedded in paraffin but formaldehyde-fixed material is to be preferred. When it is necessary to make a rapid diagnosis, Turnbull's blue reaction can be done on frozen sections and completed within a few minutes. The sections are immersed for 10 to 15 seconds in a saturated solution of ammonium sulfide. They are then transferred for a similar period into a solution made of equal parts of 1 per cent hydrochloric acid and 20 per cent ammonium ferricyanide. In such sections the blue-staining

material can be easily identified microscopically, or, if the pigment is present in sufficient quantity, there appears a blue color which can be seen grossly.

Fat is often taken up by endothelial cells derived from the segmented, compressed and disrupted vessels (Figs. 3, 5 and 11). In the present series of 67 sclerosing hemangiomas, lipoid accumulations were extensive in 12, moderate in 15, slight in 13 and absent in 27. Fifteen of the 67 specimens showed neither lipoid material nor hemosiderin.

As already suggested in the discussion of hemosiderin accumulation, it appears that the tendency toward phagocytosis on the part of endothelial cells is increased when they become isolated as the result of an increase in the surrounding connective tissue stroma. However, neither the accumulation of pigment nor the appearance of fat is necessarily most marked where the fibrosis is most advanced. Either may be completely absent in one part of a specimen and yet be prominent in nearby microscopic fields. The evidence that the lipoid phagocytes are endothelial in origin is of the same character as that for the endothelial nature of the cells containing pigment—their morphologic similarity to normal endothelial cells and the appearance of fat in cells lining patent capillaries in the lesions (Fig. 11). Further evidence that the phagocytes are identical in origin is afforded by the fact that lipoids and hemosiderin at times appear within the same cell (Fig. 3).

The nature and source of the fat is by no means a settled problem. The lipoid dissolves out in preparations which employ alcohol in the fixation, staining, or mounting of the sections. It stains an intense red when formaldehyde-fixed material is treated with scharlach R. It usually does not stain black in osmic acid preparations, and when it does so, only a small portion of the fat exhibits this reaction. It is seldom that cholesterol crystals (or clefts) can be identified. However, when these do occur, they strongly suggest that some small part of the intracellular, phagocytosed fat is related to or derived from cholesterol or cholesterol esters.

We² have regarded the fat in sclerosing hemangiomas as probably being nutritional fat; that is, fat of the same character as that found in the circulating blood under normal conditions. It is difficult to account for the large amount of lipoid material found in some sclerosing hemangiomas on any other basis. Disintegrating red blood cells have a rather high fat content. However, they are not present in amounts sufficient to contribute more than a small part of the lipoids in the examples where the fat is more abundant. It appears certain, however, that the lipoid accumulations of sclerosing hemangiomas are due to tissue reactions within the lesions themselves. Complete autop-

sies on patients with sclerosing hemangiomas have not shown any evidence of a generalized disturbance of lipid metabolism; there was no clinical evidence of such disorder in patients on whom autopsy was not performed.

The histologic sequences which accompany the process of sclerosis in hemangiomas represent regressive phenomena. Their end-result is a scar consisting chiefly of dense connective tissue. If most of the blood vessels have been obliterated and the fat and pigment have disappeared, it may be impossible to identify the original nature of the lesion. At the stages in which the accumulation of fat and pigment are so striking, the angiomatous origin of the lesions may be overlooked.

Since the sequences involved are essentially regressive, they indicate in general that the hemangiomas which undergo these changes have been present for a long time and are benign lesions. However, similar sequences of tissue behavior occur to a slight degree in portions of more rapidly growing tumors of blood vessel origin. The rate of growth is determined by the behavior of the blood vessel elements, not by the overgrowth of the stroma or the secondary tissue changes which result from it.

DISCUSSION

While no adequate statistics for the incidence of hemangiomas or sclerosing hemangiomas in different age groups are available, there is little doubt that the former are particularly common during childhood while the latter rarely appear before adolescent or adult life. It is evident that a hemangioma must usually exist for a considerable number of years before the regressive changes of sclerosis, lipid accumulation, or pigment deposition take place to any important degree. Minor degrees of these changes can be encountered in hemangiomas removed from infants and children; in general, however, the well advanced and more typical examples of the condition are apt to be found in older persons. Adequate clinical history and observation will often indicate that a hemangioma which was bright red in early years of life has subsequently faded, or has assumed a yellow or brownish color at a later time.

Sclerosing hemangiomas appear on divers parts of the body surface, but the majority (two-thirds) of them are found on the extremities. Since hemangiomas in childhood are more common on the head and neck, whereas the sclerosing hemangioma of later life is more apt to be found elsewhere on the body, it is suggested that the sclerosing processes are more prone to arise in those hemangiomas which are subjected to minor trauma. Indeed, it is conceivable that the mild but

repeated and chronic irritation of clothing on a soft and delicate structure such as a hemangioma can incite the fibrosis and other retrogressive features which have been described.

Sclerosis is not necessarily limited to those hemangiomas which are situated on the surface of the body, but it appears with greater frequency in hemangiomas of the skin. Fibrosis and lipid accumulation in these tumors have been observed in various internal viscera, particularly those of the liver. Cushing and Percival Bailey⁵ have noted similar changes in angiomas of the cerebellum; in some cases fat and pigment deposits were so extensive that the gross specimens had a vivid yellow or orange color. Orville Bailey and Ford⁶ have recorded observations on sclerosis in hemangiomas of the central nervous system—a process which is believed to be similar to the pathologic sequences here recorded for the sclerosing angiomas of the skin. In short, the secondary changes of fibrosis (or gliosis) and the accumulation of fat or pigment appear to be inherent potentialities of hemangiomas anywhere in the body.

The preoperative (or ante-mortem) diagnoses in these cases were seldom correct. The grayish color (or in some cases a lack of discoloration of the skin) combined with a firm, intracutaneous or subcutaneous mass, frequently led to diagnoses of fibroma or neurofibroma. Occasionally these lesions were thought to be sebaceous cysts. The tan or brownish variety were almost always diagnosed as pigmented nevi or melanomas. It is doubtful if any high degree of accuracy can be attained in preoperative recognition of these lesions unless there happens to be a history which suggests that they formerly had a definitely angiomatous character.

It should be emphasized again that the accumulation of hemosiderin and lipid materials are strictly localized processes and are not a part of a generalized metabolic disturbance. Sclerosing hemangiomas must therefore be differentiated from the cutaneous changes (*xanthoma cutis diabeticorum*) found in diabetes mellitus with lipemia. In this latter condition the cutaneous pathology is presumably the result of tissue reaction to extravascular deposits of lipoids. Sclerosing hemangiomas containing large accumulations of fat must also be set apart from *xanthoma planum*, *xanthoma tuberosum* and *xanthoma disseminatum*. These metabolic disturbances have been admirably discussed by Thannhauser and Magendantz⁷ and others; it is not necessary to redescribe them here, but it is important to emphasize that they are totally unrelated to sclerosing hemangiomas.

We do not wish to suggest that all xanthomas of the skin which are not a part of a systemic disease are necessarily derived from heman-

giomas. There are other localized forms of cutaneous tumors containing large amounts of fat within phagocytic cells.

In the present series of specimens there were some with giant cells which were apparently derived from endothelial cells of the angioma (Fig. 9). The finding of such giant cell xanthomas of the skin—which obviously had their origin in hemangiomas—raises the possibility that some giant cell tumors in other parts of the body may be similarly derived from hemangiomas. It is suggested that giant cell tumors of tendon sheaths develop in such a manner. Bellamy⁸ has also supported such a view; he listed as important characteristics of giant cell tumors of tendon sheaths “the presence of adult connective tissue, certain proliferating cells, multinucleated or giant cells, blood vessels with actively proliferating endothelium, and finally advanced stages of fatty degeneration.” From his studies he concluded that these do not belong among the “giant cell tumors” but that, owing to the fact that their evolution is due to proliferation of the endothelial cells of the blood vessels, they should be called endotheliomas. Garrett⁹ also concurred with this opinion. It is probable that such secondary changes in hemangiomas account for the formation of some, if not all, of the giant cell tumors of joints, which are almost entirely limited to the lower extremity and which are most frequently found in the knee. A high vascularity, accumulations of lipoids and pigment, and formation of giant cells are common findings in these neoplasms which arise from the synovia and which project into the joint cavity or extensively involve the joint capsule. Many authors, among them DeSanto and Wilson,¹⁰ are convinced that the giant cells are derived from the reticulo-endothelial system. It is not pertinent to the present communication to pursue this thesis further except to suggest that some “xanthomas” or “giant cell xanthomas” of the tendon sheaths or synovial membranes probably originate from hemangiomas which have been so altered that they are scarcely recognizable as such when the lesions are removed at operation or autopsy, unless the sclerosing process as described here is taken into consideration.

SUMMARY

Certain hemangiomas exhibit spontaneous, regressive changes which greatly alter their macroscopic and microscopic appearances. The initial step is usually dependent upon fibrosis which constricts capillary blood vessels, isolates segments of collapsed channels, and segregates groups of remaining endothelial cells. In about three-quarters of the instances, there are accumulations of lipoid material or hemosiderin, or both. In occasional specimens these substances appear to

come from hemorrhages within the angioma, but this is not true in the majority of cases. The lipid and pigment are presumably from, and extracted from, substances in the circulating blood. About one-quarter of the lesions show aggregations of endothelial cells forming foreign body giant cells. It is not surprising to find the type-cell of hemangiomas performing the dual rôle of blood vessel formation and phagocytosis since these activities of endothelial cells are well recognized in the normal reticulo-endothelial system of the body.

There is great variation in the prominence of fibrosis, vascular obliteration and phagocytic activity in sclerosing hemangiomas. When connective tissue proliferation is advanced, the lesion is quite firm, grayish, and has the gross appearance of a fibroma or neurofibroma; microscopically, the original angiomatous nature of the specimen may be largely obscured. When phagocytosis of lipid is an outstanding feature, the gross specimen may have a yellowish color and the microscopic findings may be quite similar to some of the cutaneous xanthomas, but the condition has no relationship to the generalized lipid disturbances (*diabetes mellitus*, *xanthoma disseminatum*, *xanthoma planum*, or *xanthoma tuberosum*). When pigment accumulates in high concentration, the gross lesion has a brownish color and may be mistaken for a melanoma, but the microscopic appearance of the pigment and the positive reaction to iron stains differentiate it from melanin.

The sclerosing process and the phagocytic properties may be exhibited by cavernous hemangiomas and rarely by hemangio-endotheliomas, but they are much more common in the capillary form of hemangiomas. They may be found in hemangiomas in many of the internal viscera, but they appear to be more frequent in those which are situated on the cutaneous surfaces of the body, particularly those which are located on the trunk or the extremities.

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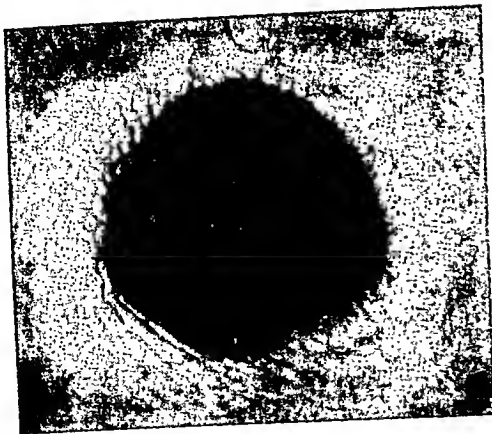
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DESCRIPTION OF PLATES

PLATE 59

- FIG. 1. Dark brown, flat, cutaneous lesion (life-sized) from a leg of a woman, 32 years old. It overlay the head of the fibula and had been present for many years.
- FIG. 2. Cross section of surgically excised specimen shown in Figure 1. Between the epidermis and the underlying subcutaneous fat is a nonencapsulated but rather well circumscribed golden brown tissue which has been formed by the deposition of lipoids and hemosiderin in a hemangioma.
- FIG. 3. Same specimen as shown in Figures 1 and 2. Camera lucida drawing from a section stained with phosphotungstic acid hematoxylin. Angioma, showing two vessels of capillary size at the right and a larger one on the left. Connective tissue takes the orange stain. A large portion of the lesion is composed of phagocytic cells filled with lipoids and lesser amounts of hemosiderin.
- FIG. 4. Same specimen as shown in Figure 3. Turnbull's blue reaction. All of the pigment stains blue, showing that it contains iron.
- FIG. 5. Same specimen as shown in Figures 3 and 4. Frozen section from formaldehyde-fixed material, stained with scharlach R and counterstained with methylene blue. The phagocytosed lipoids take an intense red stain.

1



2



E.P.H.

3



4



5

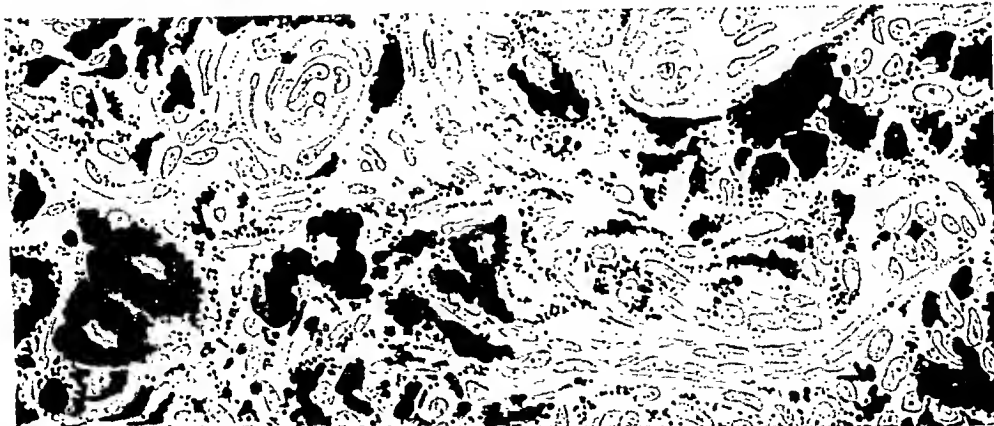


PLATE 60

FIG. 6. Sclerosing hemangioma of the lower leg of a woman, 32 years old. The lesion in the subcutaneous fat is unencapsulated. Such sclerosing hemangiomas as this are confused with unencapsulated fibromas. Eosin and methylene blue stain. $\times 17$.

FIG. 7. Sclerosing hemangioma. There is marked overgrowth of the connective tissue stroma. The capillary indicates the persistence of blood vessel elements in spite of the extent of the sclerosis. There is no pigment or lipoid in this field. Eosin and methylene blue stain. $\times 590$.

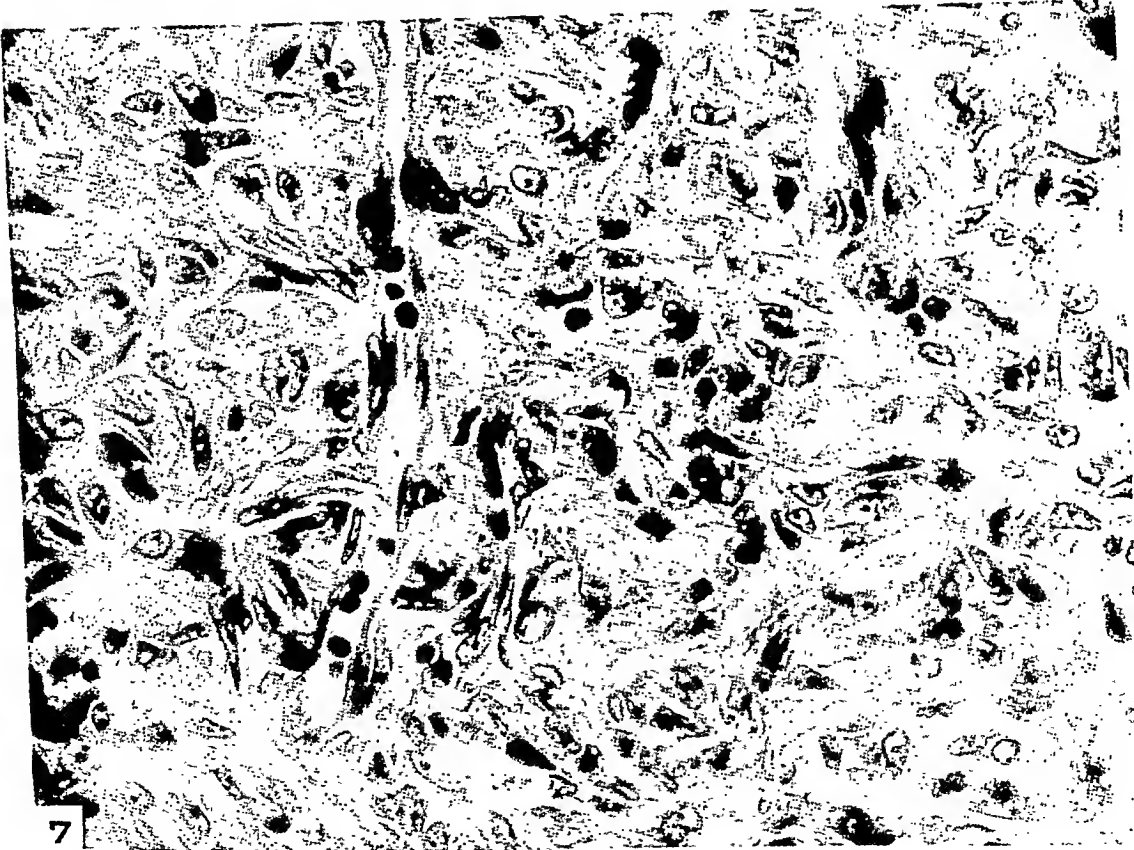


PLATE 61

FIG. 8. Sclerosing hemangioma of the lower leg. Capillary blood vessels are surrounded by a large amount of connective tissue stroma. Eosin and methylene blue stain. $\times 125$.

FIG. 9. Sclerosing hemangioma of the skin of the buttock. There are numerous multinucleated giant cells. Eosin and methylene blue stain. $\times 900$.

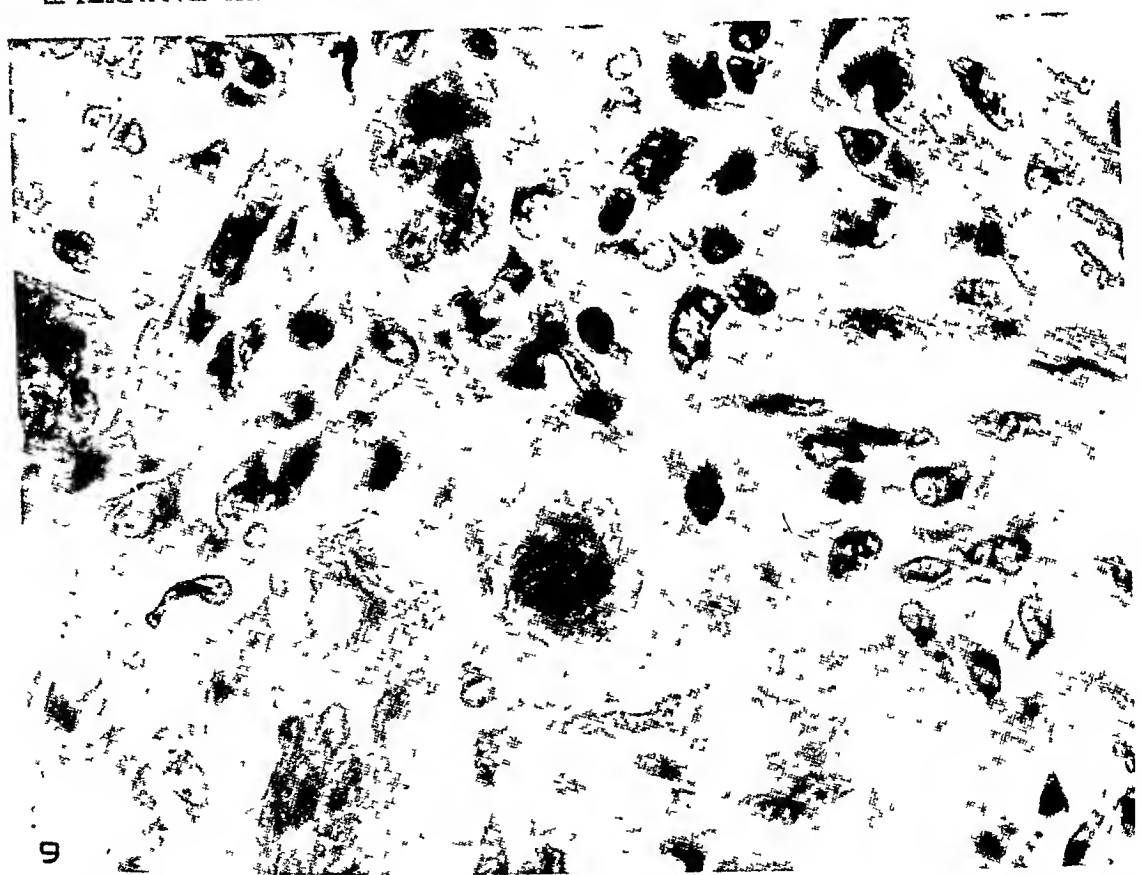
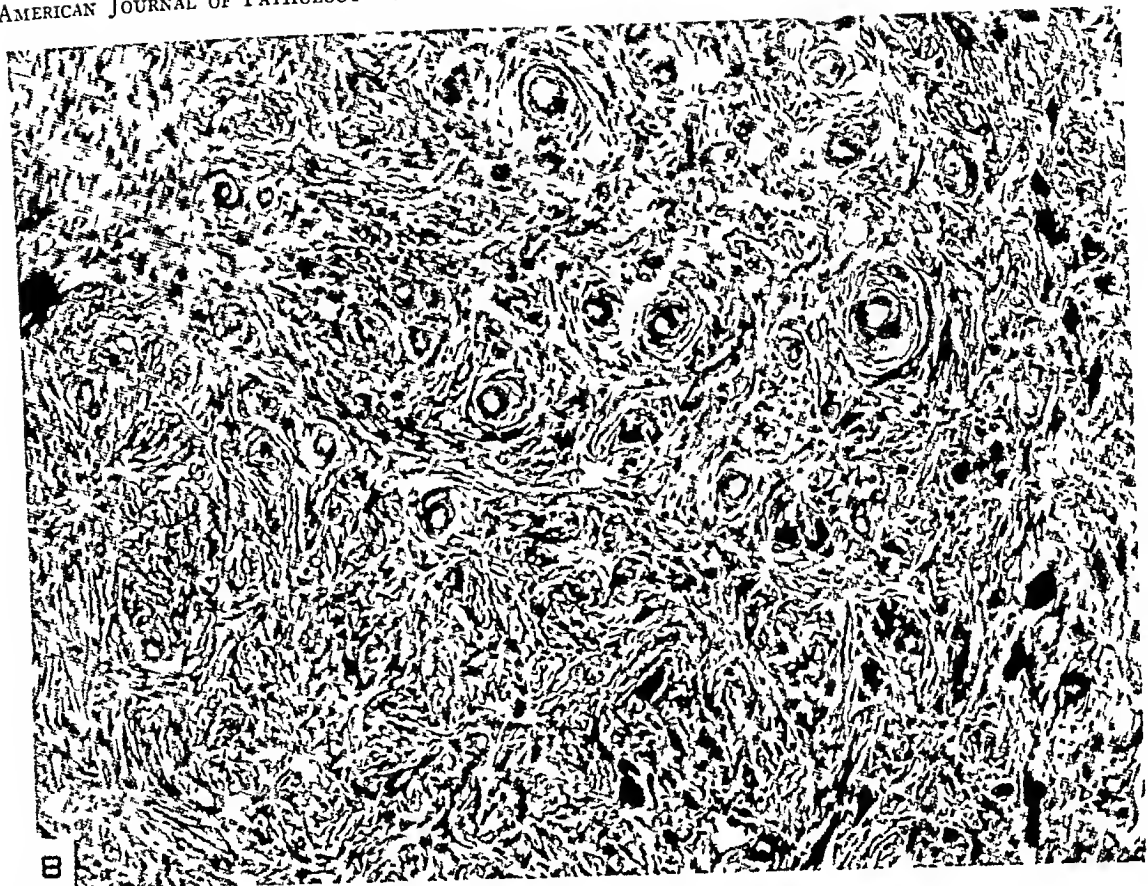


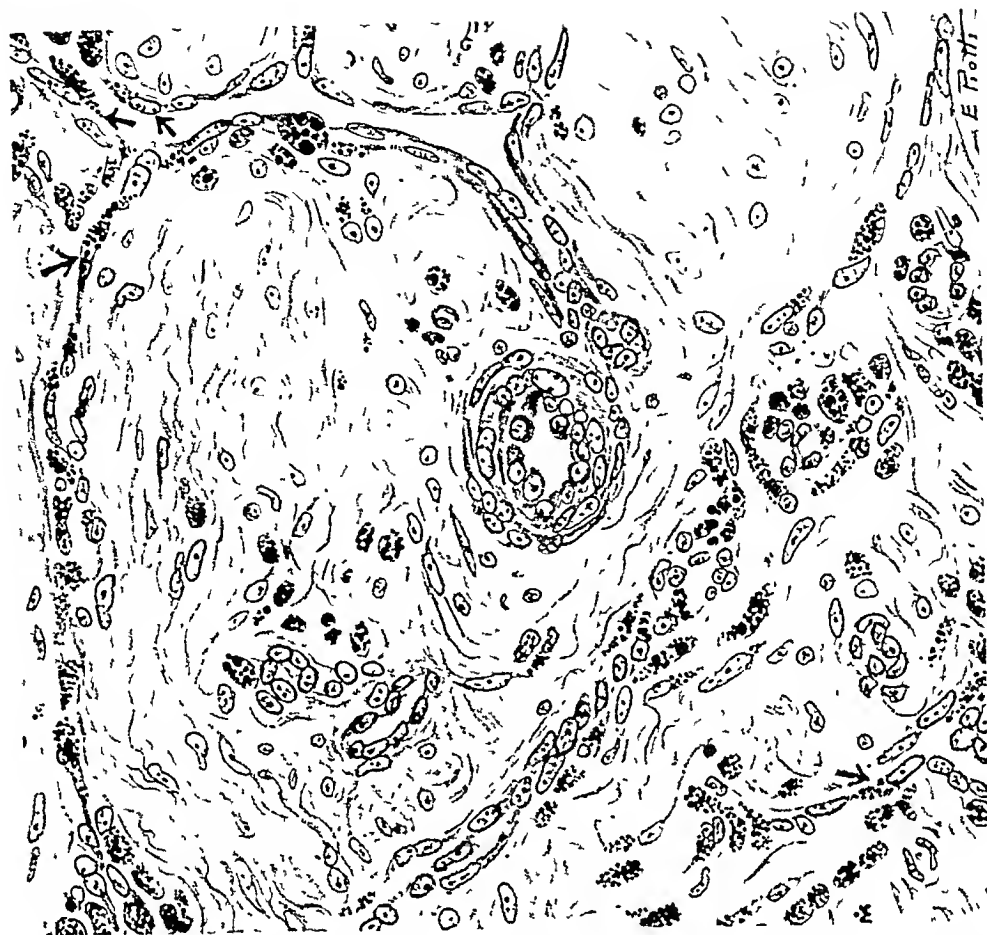
PLATE 62

The specimen drawn in Figures 10 and 11 came from a cutaneous lesion of the leg of a man, 39 years old. This lesion was brownish in color and had been noticed for many years.

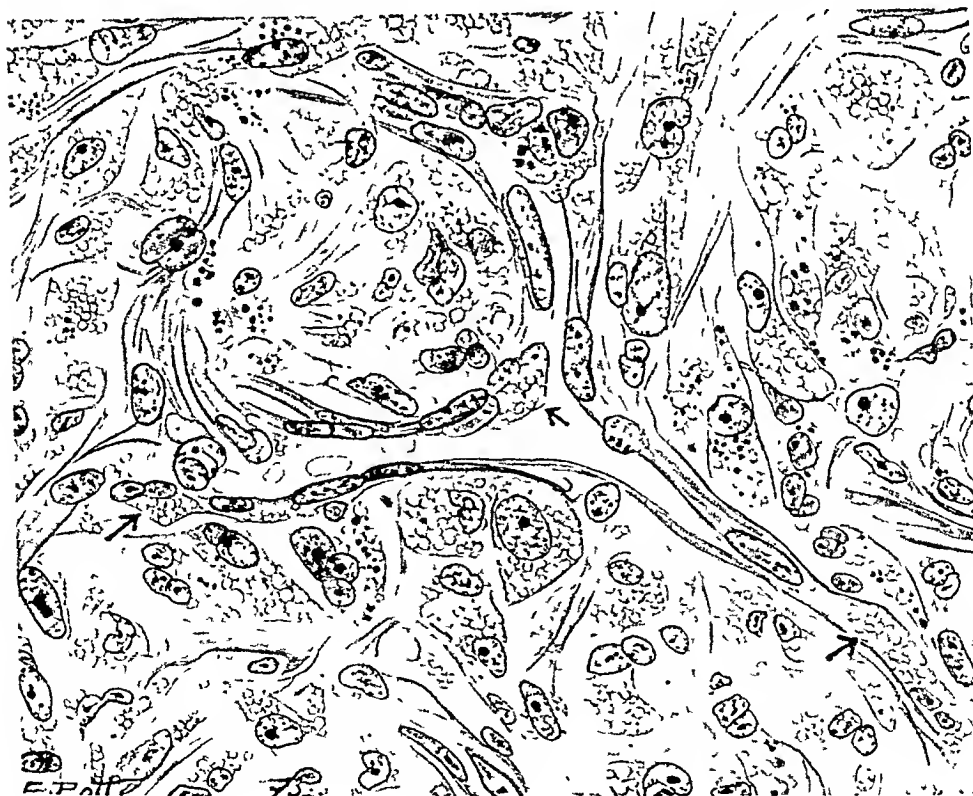
FIG. 10. Camera lucida drawing from a section stained with hematoxylin and eosin. The angiomatous nature of the lesion is shown by the formation of multiple vessels of capillary size. There is an abundant connective tissue stroma, which in some places compresses blood vessels and frequently pinches off and isolates endothelial cells. Phagocytic activity of endothelial cells is exhibited whether they are segregated or are still a part of vessel walls (arrows). The phagocytosed pigment, which is here shown in black, was a light brown in the microscopic sections.

FIG. 11. Same section and stain as shown in Figure 10, showing an area in which myriads of phagocytic cells have taken up lipoids. Phagocytic activity is also shown by endothelial cells (arrows) which are still a part of the vascular channels shown in the center of the drawing.

10



11



CHONDROSARCOMA OF BONE *

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For a while, at least in this country, there was a tendency not to single out chondrosarcoma from the general category of osteogenic sarcoma. One of the first to re-stress the need for this distinction was Phemister,¹ who did so in 1930. Now, the Committee of the Registry of Bone Sarcoma² of the American College of Surgeons, in its revised classification, has likewise accepted the concept of chondrosarcoma as an entity among the malignant tumors of bone and specifically as a lesion distinct from osteogenic sarcoma as such. This differentiation has a firm anatomic basis and is important both clinically and prognostically.

Apart from the fundamental anatomic differences between the two lesions, chondrosarcoma is by far the less common. Also, on the average, it appears at a much later age, except in comparison with osteogenic sarcoma complicating Paget's disease of bone. Furthermore, chondrosarcoma ordinarily runs a much slower course than osteogenic sarcoma, not metastasizing to the lungs for years, while osteogenic sarcoma has often already so metastasized at the time of the initial intervention (even though the roentgenographs taken at that time may still appear negative).

The basic anatomic difference between the two lesions is that chondrosarcoma develops out of full-fledged cartilage, while osteogenic sarcoma issues from more primitive tissue, developing out of bone-forming mesenchyme. Ordinarily, in an osteogenic sarcoma, most of the proliferating connective tissue, which may be quite anaplastic, becomes converted into neoplastic osteoid tissue and bone directly, though it usually also forms some cartilage, which in turn tends to undergo rapid calcification and ossification. In an occasional osteogenic sarcoma, osteogenesis may even proceed predominantly via the cartilage stage, and cartilage may thus be a prominent feature in the histologic composition of the lesion. However, even in such an osteogenic sarcoma, if one does not limit the examination to a small field of the tumor, and particularly to the periphery of it, one will also see that in other places the basic proliferating connective tissue is merging directly into neoplastic osteoid tissue and bone. On the other hand, in a chondrosarcoma, though large areas of the hyaline matrix may have become myxomatous or even calcified and ossified, the basic

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proliferating tissue of the tumor is full-fledged cartilage. In contrast to osteogenic sarcoma properly so-called, chondrosarcoma never shows neoplastic osteoid tissue and bone evolving directly from a sarcomatous stroma. These general and anatomic differences, in our opinion, constitute an adequate foundation for distinction between osteogenic sarcoma and chondrosarcoma of bone.

The understanding of chondrosarcoma is still being hindered by the idea that to make a diagnosis of chondrosarcoma on a histologic basis alone is often difficult if not impossible. Individual articles³⁻⁵ and standard textbooks^{6, 7} frequently express this idea and use it to explain discrepancies between the gravity of a given case clinically and the lack of clear-cut malignancy anatomically and especially cytologically. It is true that the histologic picture does not have to be crudely and obviously sarcomatous to indicate chondrosarcoma. However (as it is one of the main purposes of the present article to show), subtle but tell-tale histologic indications of malignancy are already present even in the early stage of the evolution of a chondrosarcoma. They may have to be searched for, particularly in an early lesion, but in any case they can be recognized if adequate material is examined and proper significance attached to relatively inconspicuous cytologic abnormalities.

Oddly enough, the literature reveals hardly any studies which adequately indicate distinctions or transition stages between benign and malignant cartilage tumors of bone on a histologic basis alone. Those articles which deal with the detailed histology of cartilage growths (see, for instance, Spuler,⁸ Ernst⁹ and Merkel¹⁰) are almost all old ones. In these, one finds attention concentrated not upon the cellular detail, but upon the intercellular matrix, and in particular upon its fibrillar structure and mucoid transformation. Actually, the findings regarding the cells, and not those regarding the intercellular matrix, are those which are significant in the evaluation of a cartilage growth.

Keiller,¹¹ in 1925, pointed out clearly the importance of attention to details regarding the cells (and particularly the cell nuclei) as a means of distinguishing between benign and malignant cartilage growths. Our own experience has taught us that a cartilage tumor is no longer to be regarded as benign if, when viable noncalcifying areas are examined, it shows, even in scattered fields: (1) many cells with plump nuclei; (2) more than an occasional cell with two such nuclei; and especially (3) giant cartilage cells with large single or multiple nuclei or with clumps of chromatin. The importance of making the correct diagnosis early resides in the relative amenability of chondrosarcomas in accessible sites to early radical surgical treatment.

In regard to many chondrosarcomas, it is possible, from the clinical course and the roentgenographic and pathologic findings, to show or deduce that they have arisen in lesions originally benign. Thus a chondrosarcoma not uncommonly issues from a solitary benign enchondroma (benign central chondroma), especially of a long tubular bone. Again, a chondrosarcoma occasionally develops out of the cartilaginous cap of a solitary osteocartilaginous exostosis (so-called osteochondroma), perhaps more commonly of a flat bone or a vertebra than of a long bone. Analogously, a chondrosarcoma may grow from one of the numerous lesions in skeletal enchondromatosis or in multiple osteocartilaginous exostoses. A chondrosarcoma which begins its development within the interior of a bone may be denoted as a *central chondrosarcoma*, and one which begins in the cartilaginous cap of an osteochondroma as a *peripheral chondrosarcoma*.

The foundation of our discussion is 15 cases recorded in our files. Of these, 10 were central and 5 peripheral chondrosarcomas. Also, a background for this study has been obtained by reviewing the cytology of the benign growths from which chondrosarcoma so often evolves. Drawing on our files, we studied 27 cases of solitary benign enchondroma and 50 cases (the last 50 of 135) of solitary osteochondroma or osteocartilaginous exostosis.

GROSS PATHOLOGY OF CENTRAL CHONDROSARCOMA

Of the 10 cases of central chondrosarcoma, there were 5 in which development out of a less serious cartilaginous lesion could still be clearly deduced and 5 in which it could not. In the former group there was one example involving the upper end of a humerus (in a girl, 19 years of age) in which the diagnosis of chondrosarcoma was based solely on the microscopic findings, the gross findings hardly giving any cue in this direction. Curettings taken for biopsy were studied originally and a resected specimen 10 months later (Figs. 1, 2, 19 and 20). The resection had been done because the lesion had been growing steadily since taking the specimen for biopsy. If it had not been for the microscopic findings in certain areas of the peripheral cartilaginous cuff of the resected specimen, we might still have regarded this lesion as a benign calcifying and ossifying enchondroma, as we did on the basis of the biopsy material. The patient has shown no recurrence during the subsequent 4½ years.*

* Our experience now includes another case in which a calcifying and ossifying enchondroma at the upper end of a humerus underwent malignant transformation. The patient was a man, 52 years of age on admission, and our original material in this case was merely some curettings for biopsy. On this basis, the lesion was at first regarded as a benign enchondroma, though subsequently we realized that we had "underdiagnosed" it. That this

There were also 2 cases of femoral chondrosarcoma presenting localized spontaneous cortical perforations as an ominous gross feature in lesions which likewise might otherwise have been regarded grossly as benign calcifying and ossifying central cartilage growths. In 1 of these, the original material was an amputation specimen from a man, 59 years of age. In the other, the material was an entire femur, obtained at autopsy from a woman of 79 years who, though she had had difficulty relating to the femur for about 4 years before death, had died from bronchopneumonia and pleurisy. (The material in this case belongs to the Erdheim collection now housed in our laboratory, and the case had previously been reported in full by Makrycostas.¹²)

In the case of the man, the amputation had been done at the junction of the middle and upper thirds of the thigh, well above the level of perforation. On cutting through the femur, the surgeon unexpectedly found the marrow cavity of the stump filled with cartilage, and, on curetting upward for several inches, also found cartilage extending above the amputation level (Figs. 3, 4 and 23). However, since permission had been given for amputation and not for disarticulation, nothing further was done at that time. Within 3 months after the amputation, a revision of the stump was done because of local recurrence, and 7 months later a disarticulation was performed because there were now large masses of tumor around the remaining portion of the femur. When the latter was dissected free, it was found to be largely enveloped by semifluctuant tumor masses of various sizes, some of which were covered merely by periosteum while others also had muscle adherent to them. Coronal section of the specimen revealed that except at the end of the stump the medullary cavity was filled with soft, whitish, and obviously cellular neoplastic tissue, extending up into the spongiosa of the metaphysis and part of the neck. Furthermore, one could see that the cortex of the shaft was permeated everywhere by tumor and that there were large pockets, bordered by modified and eroded cortex and distended periosteum and filled with soft, semifluid, or gelatinous neoplastic cartilage. From the gross appearance there could not be the slightest doubt that one was dealing with a chondrosarcoma. (For the microscopic picture at that time, see

was so was borne out by the fact that within 12 months the lesion had become flagrantly malignant even clinically. The upper end of the humerus was now resected and showed a chondrosarcoma which had erupted from the bone in one region. Especially where this was the case, the neoplastic tissue was no longer cartilaginous but rather of a fibrous nature and gray-white in color. It appeared histologically as a rather anaplastic, collagen-forming, spindle-celled sarcoma but showed no ossification. From such areas alone, it would be difficult to surmise that the tumor had developed from an enchondroma. Elsewhere, even in the original calcified and ossified cartilaginous portion of the tumor, there was evidence of activated growth of the dormant cartilage cells.

Figure 24). At present, about 2 years after disarticulation, the patient shows another recurrence, with bulky tumor masses in the groin and also in the pelvis.

There were 2 cases in which the chondrosarcoma was engrafted upon lesions of Ollier's disease (skeletal enchondromatosis). In 1—that of a woman of 56 years—the left lower limb had been bumpy, stubby, and shortened from birth, and its bones (especially the femur and tibia) were filled with cartilage plugs and nodules of widely varying size. Some time before this limb was disarticulated, a perforation had occurred through the cortex of the lower end of the femur medially, and a large cartilaginous tumor mass of gray gelatinous tissue, continuous with the interior of the bone, had developed in the soft parts of the lower half of the thigh, particularly in front. It was in this area that the chondromatous tissue had undergone transformation into a chondrosarcoma, while, in contrast, the cartilage filling the upper half of the femur and all of the tibia showed nothing suggestive of malignant change.

The other case was that of a boy, 19 years of age, whose enchondromatosis seemed to be limited to the bones of the right hand and forearm. In this case, the distal end of the ulna was found distorted by a protuberance measuring approximately 6 by 5 by 5 cm., and the affected area was resected. Both longitudinal and transverse sectioning of the specimen showed that the distortion had been created by a mass of cartilage which was continuous with cartilage in the interior of the ulna, the cortex being defective over most of the region where the mass was situated (Figs. 5 and 6).

We turn now to the 5 cases of central chondrosarcoma in which, at the time of initial examination, the pathologic material yielded no evidence that the sarcoma had evolved out of a less serious central cartilaginous lesion. In these cases, the cartilage growth may have been malignant from its inception. In one instance, that of a man of 39 years, an amputation specimen, presenting a lesion (Figs. 8, 9, 10 and 18) in the lower end of a femur, showed a flask-shaped distention of the affected region, 10 cm. wide, 12 cm. deep and 15 cm. long. The amputation level definitely cleared the tumor so far as the interior of the bone was concerned, but apparently did not clear it in relation to the soft parts and periosteum. Within 6 months after the operation, the femoral stump showed extensive rarefaction and cortical destruction, extending upward for about 10 cm., and there were also evidences of recurrence of the tumor in the soft parts about the stump. Disarticulation was now done. On dissecting the muscles and soft tissues about the femur, whitish, glistening tumor was found to be extending

into the muscles and fat. A sagittal section of the stump showed neoplastic tissue extending 7 cm. up the medullary cavity and directly continuous, through defects in the cortex, with that in the surrounding muscles and fat. About 4 cm. above the main tumor mass, another nodule of tumor, 3 cm. in its longest diameter, was found in the marrow cavity of the stump, the intervening part of the cavity apparently being uninvolved.

We have 1 case of chondrosarcoma developing apparently *de novo* in a short tubular bone, specifically, the basal phalanx of a finger (a rare location). The lesion was as large as a child's fist and the skin was movable over it. The tumor had destroyed most of the phalanx, sparing only the basal part. Indeed, even this part showed ingrowth of a few neoplastic nodules. The tumor tissue was typically lobulated, had zones of cystic softening and hemorrhage, and was sharply delimited.

A somewhat different gross picture (Figs. 7 and 17) was presented by a chondrosarcoma in the lower end of a femur of a man, 29 years old. Though the spongiosa in the affected area was filled with tumor, the general contour of the bone itself in that area was not much modified. However, the whole lower third of the thigh was tremendously enlarged, since the tumor had erupted through the cortex and had grown exuberantly in the soft tissues about the lower end of the femur. The site of eruption of the tumor into the surrounding soft tissues was the thin cortex just above the condyles. This gross picture (early penetration of the cortex of the affected area and lush growth of the tumor in the overlying soft tissues, without much distention of the affected bony part) is nearly always seen in chondrosarcomas starting within a flat bone. Our records include 2 such cases, in both of which the lesion originated in an innominate bone.

In 1 of these cases—that of a man of 31 years—the tumor, starting in the left pubic bone, presented on the outside of the pelvis as a large, lobulated, cystic cartilage mass extending along the inner side of the thigh to the perineum. On the inside of the pelvis the cartilaginous mass produced a tumor which, by rectal palpation, could be found extending to the prostate. The gross material from this case consisted of numerous small, irregular fragments of cartilage, most of which were firm and of a homogenous, glassy, blue-white appearance, though a few were softer and more yellowish, apparently in consequence of degenerative changes. None of the cartilage showed areas of calcareous impregnation and ossification such as one would find if he were dealing with a peripheral chondrosarcoma developing from the cartilaginous cap of an osteochondroma.

In the other case—that of a man of 40 years—the tumor seemed to have arisen in the left acetabulum, had broken into the hip joint, involved the homolateral head of the femur, ischium, and pubic bone, and also formed an elastic tumor mass which spread in the pelvis toward the prostate and overlay its left lobe. Complete removal of the tumor was obviously impossible, but many bits of hyaline and myxomatous cartilage were removed for examination. The patient died about 5 months later at another hospital, and, at autopsy,* a large local tumor mass was found, as well as tumor nodules along the aorta and in the right adrenal, pleura, lungs and heart.

In general, chondrosarcomas of the pelvic region may attain truly fantastic size. This is favored by the difficulties of their radical treatment in this region, the high resistance of chondrosarcomas to irradiation therapy, and the likelihood of delay in the appearance of distant or strategic extensions or metastases. Indeed, an innominate bone which is the site of such a chondrosarcoma not infrequently comes to be embedded, over a considerable area, in a mass of neoplastic tissue 12 inches or more in diameter. In addition to breaking into the hip joint and involving the upper end of the femur, it may even come to involve heavily the sacrum and lumbar vertebrae.

GROSS PATHOLOGY OF PERIPHERAL CHONDROSARCOMA

As noted, "peripheral chondrosarcoma" is here being used to mean only a chondrosarcoma which starts its development in the periphery (as contrasted with the interior) of a bone, and specifically in the cartilaginous cap of an osteochondroma. Of the 5 pertinent cases on which we have anatomic material and clinical data, 4 apparently were instances of solitary osteochondroma with transformation into chondrosarcoma, while the other was an instance of multiple osteochondroma (multiple osteocartilaginous exostosis) in which one of the outgrowths had undergone malignant change. To judge from our experience (and also from the literature), a solitary osteochondroma seems to undergo malignant degeneration only rather rarely, relatively speaking, when one considers the frequency of solitary osteochondroma among the tumorous bone disorders. The incidence of malignant degeneration of one or more of the osteochondromatous outgrowths in cases (themselves more rare) of multiple osteocartilaginous exostosis seems to be higher, on a case-to-case basis. This impression likewise

* We are indebted to Dr. Samuel H. Rosen of the Laboratory of Pathology, Montefiore Hospital, New York City, for the opportunity of examining the protocol and studying slides from the autopsy material in this case.

appears to be borne out by the literature, which reveals a considerable number of such cases.*

In 1 of our cases (that of a woman of 44 years), the chondrosarcoma developed out of a solitary osteochondroma of the left fourth rib, in which a slowly enlarging tumor mass had been known to be present for 12 years. In the extirpated piece of rib the sternal end was found encased, particularly anteriorly, in a mass of lobulated tissue (Figs. 11, 12 and 21). This tumor mass measured 4 by 3.5 by 2.5 cm. and was covered by a fibrous capsule. It felt firm and elastic in some places and osseous in others. When the patient was seen 3 years later, there was a recurrence at the site of the original operative intervention, the tumor mass now being about three times the size of the original lesion. A wide resection was done, and the neoplastic tissue of the recurrent growth was found composed of large facets of cartilage still showing some evidences of calcification and ossification, especially centrally. On microscopic examination, it also showed (Fig. 22) progression of its malignancy cytologically beyond what was apparent in the original specimen. Five years after resection of the recurrent lesion, Mayer¹³ reported that there had been no further recurrence in this case.

In a case of costal chondrosarcoma developing in association with multiple exostoses, it was the left sixth rib that was affected, with an almost grapefruit-sized tumor in the midaxillary line (Fig. 13). Exploration showed that the mass overhung and separated the adjacent fifth and seventh ribs. It was also found that anteriorly a few small chondromatous nodules had penetrated the capsule and were separate from the main mass, and that posteriorly the tumor had penetrated the pleura. The mass was resected (though not in one piece), and its gross appearance was again found typical of peripheral chondrosarcoma. Specifically, the interior of the tumor showed a rather heavy sprinkling of bone and foci of calcification among islands of cartilage. Externally, there was an irregular zone of cartilage several centimeters thick in some places and clearly delimited here and there from the interior of the tumor by a line of endochondral ossification.

Within 4 months, this patient showed a local recurrence, and a wider local resection of the chest wall was done at another institution. At

* Since this article was submitted, we have studied systematically our cases of hereditary multiple exostosis. We found that, of the 28 relevant cases, 3 (including the one just mentioned above) had come to show malignant transformation of one or another of the exostoses. However, even this incidence of nearly 11 per cent probably does not represent the true incidence of this complication in this series of cases. All 3 subjects were adults when the malignant growth began to be manifest. Since the great majority of the other patients in our own series of multiple exostosis cases were still children or adolescents, one must allow for the possibility that later in life some of these, too, might develop chondrosarcomas.

this operation the tumorous cartilage was found to be practically without the calcification or ossification so typical of peripheral chondrosarcoma, at least in the original specimen. Several months later there was again evidence of recurrence. The patient died almost 2 years after the original operative intervention, and 4 years after he had first noted the presence of the tumor. At autopsy,* considerable gelatinous cartilage was found beneath the soft tissues of the chest wall. Adherent to the pleura, there was a mass of the same type of tissue, practically filling the entire pleural cavity on the affected side and encasing and collapsing the lung (Fig. 25). There were no visceral metastases. The autopsy confirmed the presence of numerous cartilaginous exostoses on various bones, thus establishing the diagnosis of multiple exostoses already made earlier on a roentgenographic basis.

The case—that of a man of 46 years—in which the chondrosarcoma developed from an osteochondroma on a tibia is interesting in that the patient had been aware for 24 years of a hard lump at the upper and outer margin of this bone. During this time the lesion had grown only very slowly, but, about 2 months before the patient's admission to the hospital, it took on a spurt of growth, apparently after local trauma, and became increasingly painful. On admission a large, hard, bony mass, 6 inches in diameter, was palpable on the anterolateral margin of the tibia 1 inch below the tubercle; and on the anteromedial border, about 3 inches below the joint, a smaller mass was palpable (Fig. 14). At operation it was found that the tumor, which had arisen from the tibia, lay both anteriorly and posteriorly to the interosseous membrane. The neoplastic tissue was removed piecemeal, but not all of it could be removed. Nevertheless, a quart (0.95 liter) of material was obtained and submitted for pathologic examination. Grossly, by piecing the bits together, one could see, as for the tumors of ribs just described, that centrally in the tumor mass there was much calcification and ossification and that peripherally there was an undulating, wide cap of cartilage containing streaks of calcification. About 5 weeks later, the limb was amputated above the knee joint, and during the ensuing 2 years the patient has been free from any evidence of recurrence.

The findings in our other 2 cases of peripheral chondrosarcoma are essentially in line with those already indicated. One was a case of tumor of the left innominate bone occurring in a woman of 30 years who dated her difficulties from a fall 8 years before admission to the hospital. On admission, a huge tumor was palpable on the iliac portion

* We are indebted to Dr. Milton Helpern of the Medical Examiner's Office of New York City for permission to view the autopsy and obtain details of the pathologic alterations in this case.

of this bone. The other case was that of a woman, 59 years of age, in whom the lesion was in the left scapula. Although the scapula had been converted into a lobulated mass of the size and shape of a small football, it was because of neurologic symptoms referable to pressure on the brachial plexus that the patient was admitted to the hospital.

EXTENSION AND METASTASIS

Even if left to themselves, chondrosarcomas (central or peripheral) are likely to remain only locally invasive for years. After an initial surgical intervention, a local recurrence is certainly to be expected if the tumor has not been widely excised. Local recurrence of the lesion in even bulkier form is almost the rule at subsequent interventions under these circumstances. However, even then there may still be no definite spread of the growth for a long time, and death may take place from other causes. When a chondrosarcoma finally does spread, the tumor tends to break into the regional venous channels, and, by intravascular growth and extension, without necessarily adhering very much to the vessel walls, may reach the heart and lungs. Though it is ordinarily years before this occurs, in an occasional case the disorder runs its course so rapidly that the patient is dead within some months after the original tumor in the bone was first noted. The presence of severe respiratory and cardiac difficulties for a time before death in a patient with a chondrosarcoma may well be a clinical indication that cordlike intravascular growth and extension of the tumor to the heart and lungs has taken place.

A remarkable example of such neoplastic extension is the case, described by Ernst,¹⁴ of a chondrosarcoma involving the lower part of the vertebral column. In this case there were tumor plugs in both the renal and the suprarenal veins, the left internal spermatic vein, the azygos vein, the inferior vena cava, the right auricle, and the branches of the right and left pulmonary arteries; and still the pulmonary parenchyma was free from metastases. Kósa¹⁵ described a femoral chondrosarcoma with neoplastic extension to the homolateral femoral and iliac veins, the inferior vena cava, the right heart, and both branches of the pulmonary artery, even to the capillaries, again without the occurrence of any parenchymal metastases, even in the lungs.

In other cases, while extending into the large venous channels, the tumor also gives rise to parenchymal metastases, at least in the lungs. In the case described by Weber¹⁶ there were metastases not only in the lungs but also in the liver (around tumor-plugged branches of the portal vein), but in the case reported by Warren¹⁷ the metastases were limited to the lungs. Actually, metastases elsewhere than in the lungs

are uncommon in connection with chondrosarcomas. The possibility of lymphatic spread also exists, and extension of the tumor to lymph nodes, especially regional, has occasionally been reported. However, it is to be recognized that small secondary nodules of tumor not far from the primary mass might easily be wrongly interpreted as representing lymph nodes which have undergone complete replacement by tumor.

MICROSCOPIC PATHOLOGY

As noted, we used as a background for comparison in our study of chondrosarcoma 27 solitary benign enchondromas* and 50 solitary osteochondromas or osteocartilaginous exostoses. In regard to the enchondromas, apart from their gross features, it was found that they varied considerably in respect to cellularity, some being relatively rich in cells, others relatively poor, and still others showed intermingled areas of greater and lesser cellularity. (On the average, of course, they were definitely less cellular than chondrosarcomas.) Where the intercellular matrix was hyaline, the cells of enchondromas were usually roundish, but where the matrix appeared edematous or even mucoid, they tended no longer to be so, and their cytoplasm was likely to be multipolar or stellate. Some lesions or parts of lesions showed considerable or even heavy calcification of the intercellular matrix, and even some osseous metaplasia of heavily calcified areas.

However, it was found that in defending the benignity of an enchondroma, no great weight was to be attached to the relative cellularity, the distribution and shape of the cells, the presence or absence of lacunae about the cells, or the amount and character of the ground substance between the cells. Indeed, it became apparent that the picture of benignity is created by the characteristics of the cells themselves (as observed in viable and noncalcifying areas of the lesion) and specifically by the following features: (1) the component cartilage cells are fairly consistently rather small; (2) the vast majority of them have only one nucleus; (3) this nucleus tends strongly to be rather small and definitely not plump in relation to the cell as a whole; (4) such cells as are binuclear usually are still small, still have small (and also not plump) nuclei, and are found only occasionally and even then only in scattered fields; and (5) in particular there are no large or giant cartilage cells with large single or multiple nuclei or with clumps of chromatin. (See Figures 15 and 16, and compare with Figures 17, 18, 20, 24 and 25, for instance.)

* Since the present article was submitted, our material on the benign central cartilage growths has been published. See: Jaffe, H. L., and Lichtenstein, L. Solitary benign enchondroma of bone. *Arch. Surg.*, 1943, 46, 480-493.

In regard to the osteochondromas, it is, of course, the status of the cartilage cap that is significant. This cap may or may not be still actively growing at the cartilage-bone junction and increasing the size of the osteochondroma at this site. Apart from the zone in which endochondral growth may thus be going on, and apart from areas which are undergoing calcification and ossification, the condition of the cartilage cells of the cap will be essentially like that noted as obtaining in benign enchondromas. If a growth zone is present in the deep part of the cap, the cartilage cells in that zone will be found lined up in short columns, enlarged, and even presenting significant numbers of plump, single or double nuclei. However, the whole cellular picture in such a zone of growth is an orderly one, corresponding to that seen, for instance, at the growing surface of an epiphyseal cartilage plate or in relation to cartilaginous callus, and hence not needing to be considered in judging the condition of the cap as a whole in respect to benignity. Indeed, in any focus of cartilage proliferation (for instance, even in a cartilaginous joint mouse) one can expect to find some cells of the type which, if observed in a cartilage tumor of bone, would make one view the lesion rather seriously. However, what we are saying about the cytologic diagnostic criteria applies, of course, only to the cartilage tumors.

In evaluating the cytology of a chondrosarcoma, as in evaluating that of an enchondroma or an osteochondroma, attention should likewise be concentrated solely upon areas which are viable and not heavily calcified or ossified. The reason is that in areas which are undergoing necrosis, heavy calcification, or ossification, the cartilage cells (and particularly their nuclei) are likely to be swollen because of these changes, that is, for reasons irrelevant to the benignity or malignity of the lesion. A chondrosarcoma may violate the histologic criteria of benignity to such an extent that even without knowing anything about the gross appearance of the lesion the examiner becomes aware of its malignity at a glance (Fig. 25). On the other hand, a chondrosarcoma may violate the criteria rather subtly and require, for its proper diagnosis, detailed scrutiny, under high magnification, of many microscopic fields, and more material than is ordinarily obtained for biopsy by a punch (Figs. 20, 21 and 23). When this is the case, the lesion is often "underdiagnosed" histologically, at least at first, as a benign enchondroma or osteochondroma, its chondrosarcomatous nature not being appreciated until a recurrence appears and perhaps reappears. We ourselves had originally "underdiagnosed" a number of our cases, as was borne in upon us by subsequent sad experience with them.

The case (Figs. 1 and 2) in which the lesion was in the upper end

of a humerus represented an instance of very early and subtle deviation in the direction of malignancy. Histologically, on the basis of the original biopsy, one could not have entertained, in this case, any other diagnosis than that of a benign enchondroma (Fig. 19). The cartilage of the resected specimen obtained 10 months later showed, especially in parts of the peripheral zone, an unequivocal change in the direction of malignancy. Specifically, as compared with those of the specimen taken for biopsy, the cartilage cells in these areas, though small on the whole, had plump nuclei. Furthermore, some fields, here and there, showed a significant sprinkling of cells with two or even four nuclei, and also a number of large cells, each of which had a large nucleus (Fig. 20).

The rather small peripheral chondrosarcoma developing out of an osteochondroma of a rib (Figs. 11 and 12) again shows the necessity of searching for, and paying close attention to, cytologic abnormalities which may not be obvious at first sight. In the wide cartilaginous cap the cells in this lesion, too, were, on the whole, somewhat plump, and in some areas more than a sprinkling of them were binuclear, while here and there some definitely large ones with single large nuclei were also encountered (Fig. 21). In the recurrence studied 3 years later, the lesion showed unmistakable histologic evidences of progression in malignity. Almost everywhere, but more definitely in some fields than in others, the lesion was very cellular and the cartilage cell nuclei were now plump, on the whole. Many more binuclear cells and many more large cartilage cells with single large nuclei were seen. This was the picture not only at the periphery of the recurrent lesion but to a very great extent in the cartilage lying among calcified cartilage and osseous material in the interior of the lesion.

In the case illustrated in Figures 3 and 4, ominous cytologic features were recognizable though not conspicuous in many areas of the amputated specimen, including curettings from the marrow cavity of the femoral stump (Fig. 23). They were very prominent in the disarticulated femoral stump, obtained 10 months later, which showed flowering of the lesion into an exuberant chondrosarcoma (Fig. 24).

Eventually, in a fully developed chondrosarcoma, central or peripheral, the neoplastic tissue is richly cellular. In addition, it shows striking irregularity in the size of the cells and their nuclei, the presence of numerous plump cells with multiple nuclei, pronounced hyperchromatism of the nuclei, and the presence of many uninuclear giant cells (Fig. 25). From lesion to lesion there are variations in detail. However, if material taken at different times is examined, the general cytologic picture of that particular lesion tends to remain more or less

consistent, though one can usually trace cytologically the progression of malignancy from one examination to another.

In the histologic diagnosis of chondrosarcoma, not too much importance should be attached to the scarcity or even absence of mitotic division figures. If one concentrates attention upon these, one may miss the diagnosis, since cell division in chondrosarcomas tends to be amitotic. This does not mean that advanced chondrosarcomas may not show more than a sprinkling of mitotic division figures, but when they do, the histologic diagnosis is already obvious for other reasons.

Partial or extensive alteration in the character of the cartilage matrix—that is, change from hyaline to myxoid or mucoid—is not a particularly significant element in the composition of chondrosarcoma. It is true that, especially in the more bulky growths, larger or smaller areas of semisoft or gelatinous cartilage are likely to be found. However, these represent merely a nonspecific, secondary degenerative change whose incidental character is usually attested by the presence elsewhere of areas of hyaline cartilage. Chondrosarcomas which show extensive myxomatous degeneration are often designated as myxochondrosarcomas. It seems to us, however, that the prefix *myxo-* might well be dropped, even in these cases, since there appears to be no special reason for this emphasis on an aspect of the pathologic picture which is not distinctive and which has nothing to do with interpretation of the lesion in respect to malignancy.

The presence, in a cartilaginous growth, of some, or even considerable, calcification and ossification is not inconsistent with its being a chondrosarcoma. In a central chondrosarcoma, such areas may be regarded merely as evidence that the growth had been benign in the past and that it had matured and regressed to some extent at some time before undergoing revivescence and malignant transformation. In a peripheral chondrosarcoma, some calcification and ossification of the matrix, at least in the early phases of the growth, are to be expected. They should not, though they often do, lead one to designate the lesion as a chondro-osteosarcoma or an osteochondrosarcoma and thus to imply that it represents a form of osteogenic sarcoma. Even if an osteochondroma is tending toward malignancy, it is in the nature of its proliferating cartilage cap to undergo calcification and ossification in the course of its development.

In relation to the microscopic diagnosis of chondrosarcoma, some consideration should be given to a tumor which we¹⁸ have recently discussed under the heading of "benign chondroblastoma of bone." This tumor has usually been referred to as the "benign calcifying," or "epiphyseal chondromatous" giant cell tumor. It requires mention here

because it has sometimes also been regarded as a chondroblastic sarcoma or as a chondrosarcoma. As we have pointed out, however, though there is an aura of cartilage about the benign chondroblastoma, the latter is to be regarded as a specific type of tumor which cannot correctly be interpreted as a tumor of mature cartilage at all, and certainly not as a sarcomatous cartilage growth. The tumor cells of this lesion, which do not mature into full-fledged cartilage cells, should be interpreted, in our opinion, as chondroblasts, and the tumor itself as a chondroblastoma. The word "benign" was prefixed to indicate that the lesion is not a malignant one, despite its richly cellular character. Benign chondroblastoma starts its development in an epiphysis, usually of some long bone. It seems to predilect males, and the patients, in contrast to those with chondrosarcoma, are almost always adolescents or post-adolescents. The lesion is entirely benign and heals without recurrence after thorough curettage, even without supplementary radiation.

We have seen 3 examples of another lesion which seems also to require mention here but which we have great difficulty in classifying. It starts presumably as a focus of more or less collagenous connective tissue which undergoes myxomatous degeneration, and lends itself rather easily, even histologically, to confusion with chondrosarcoma. In 2 of the cases, the lesion was in a femur, at the lower end of the shaft, and in 1 in the proximal end of the shaft of a metatarsal bone. In all 3 cases, the lesion tended to be eccentric, bulged out the cortex in its vicinity, and appeared roentgenographically as a roundish, moderate-sized area of trabeculated rarefaction. Histologically, study of many fields from such a lesion shows residua of collagenous spindle-cell connective tissue in process of myxomatous degeneration (Fig. 26). In the clearly myxomatous areas, the cells show branching cytoplasmic processes and are separated by larger or smaller amounts of myxomatous basophilic cytoplasm. Some of these cells may even show retraction of these processes and come to lie in lacuna-like spaces. Particularly where the collagenous connective tissue is actively undergoing myxomatous degeneration, cells with very large hyperchromatic nuclei and cells with two, three, or more such nuclei may be numerous, and create the impression of tumor giant cells. In these areas, polymorphonuclear leukocytes are also to be seen. These lesions may also show areas of cystic degeneration and residua of hemorrhage, indicated by the presence of some foreign body giant cells and macrophages laden with hemosiderin.

Among the cases which Bloodgood¹⁹ has described under the heading of "myxoma of bone" we find one in which the histologic picture

as reproduced photographically is exactly like that in these 3 cases which are puzzling us. In Bloodgood's case, there were a number of recurrences after curettage, thermal cautery and irradiation therapy, but though he gives a very somber prognosis in general for the lesion which he calls "myxoma of bone," the patient in this particular instance was still living and without metastasis more than 2 years after the original surgical intervention. In regard to our own cases we know that in spite of the ominous impression created by the histology of the lesion, mere curettement of it in two instances, and resection of it in one, was not followed by recurrence during a subsequent period of 2 years, 1 year, and $3\frac{1}{2}$ years, respectively. We, ourselves, are not yet clear as to the proper classification of the lesion, and wish merely to record our findings in regard to it because of its superficial resemblance to chondrosarcoma.

CLINICAL ASPECTS

Age and Sex Incidence, and Localization

Most of the patients with chondrosarcoma have reached adulthood. In occasional instances, they are still in the teens, but in the great majority they are between 30 and 50 years of age. The disorder does not seem to predilect either sex. These generalizations, drawn from our relatively few cases, seem to be in line with what one can gather from the literature. In evaluating the latter, however, one must make due allowances for misclassification of cases. Many cases reported under the heading of chondroma turn out, on closer study of the report, to represent what is paradoxically labeled "recurrent chondroma" or "benign metastasizing chondroma," that is, actually chondrosarcoma. These considerations make it difficult also to estimate, from large numbers of cases, the true incidence and localization of chondrosarcoma. It appears, however, that the long tubular bones, the innominate bones, and the ribs, in descending order, are the most common sites.

Clinical Findings

In most of our cases the patients had already had a long but not dramatic history at the time of admission. This was true irrespective of the bone affected and of the central or peripheral origin of the chondrosarcoma. On the whole, the history tended to be longer in the cases of peripheral chondrosarcoma. Very short histories, associated with a fulminating clinical course, are clearly exceptional (though a few have been recorded) and we ourselves have observed no cases of this kind.

Thus in the case of peripheral chondrosarcoma which evolved from

a costal osteochondroma, the patient stated that for 12 years she had been aware of a painless mass which slowly grew to the size of an egg. In the case in which the chondrosarcoma (a huge one) developed from an osteochondroma of a tibia, the patient stated that for 24 years he had been conscious of a slowly enlarging, but certainly not disabling, tumor mass in the upper part of the leg. In our case of peripheral chondrosarcoma of an innominate bone there was an 8-year history of a slowly progressive tumorous enlargement associated with scarcely any clinical difficulty. Even in the case of multiple exostoses in which the chondrosarcoma developed on a rib, 2 years elapsed before the lesion had reached the size of a grapefruit and the need for treatment became urgent.

While, as noted, the histories in the cases of central chondrosarcoma tend to be somewhat shorter, yet it is not unusual, in such cases either, to get a history dating back 4 or 5 years. For instance, in one of the cases of central chondrosarcoma of a femur the patient stated that he had had dull, aching, local pains at intervals for 5 years, with some short-lived exacerbations. It was not until he accidentally palpated a mass along the lateral aspect of this femur that he sought medical care for his difficulty. On the other hand, in another case in which the chondrosarcoma was in a femur, the complaint was of only 6 months' standing. This patient gave a history of pain and functional disability in the region of the knee, worse at times, but never bad enough to confine him to bed. In one of the cases of central chondrosarcoma of an innominate bone, there was a history that for 5 years there had been moderate and intermittent pain and disability in the region of the homolateral hip joint, latterly associated with pains shooting down the entire leg. Local physical examination revealed the enlargement, slight to considerable, of the area affected. The enlarged part was firm or bone-hard, and usually not very tender, and the overlying skin was never red or warm. When the area involved was near a joint, the latter was likely to be found somewhat swollen and its motion somewhat restricted.

Trauma

We analyzed our cases as to the possible relation of a trauma to the development of the tumor. It is significant that in about half of the cases in which the data were adequate the patients stated definitely that they knew of no antecedent trauma which could have any bearing upon the lesion. Two patients in whom the chondrosarcoma developed in an osteochondroma stated that 24 and 8 years before, respectively, trauma led to discovery of a local tumescence which must have been

the osteochondroma. Whether this long-antecedent trauma instigated growth and transformation of the osteochondroma into a chondrosarcoma cannot be definitely known. Our cases include one osteochondroma undergoing slow transformation into a chondrosarcoma clearly without the agency of trauma. In fact, there was only 1 case among our 15 in which it was reasonable to suppose that trauma might have been a factor. This was the case of a man suffering from multiple exostoses who received a blow to one side of his chest and who, 6 months later, showed a definite and enlarging malignant tumor of a rib in the general region of the trauma. It should be borne in mind in this connection, however, that the natural tendency toward malignant transformation of an osteochondroma is stronger in cases of multiple than in those of solitary osteocartilaginous exostoses. Altogether then, even in those cases in which there seems to be a traumatic factor, it cannot be accepted without mental reservations.

Roentgenographic Findings

These may go far toward confirming the suspicion, perhaps already aroused by the history and physical findings, that one is dealing with a chondrosarcoma. Certainly, a long bone presenting an irregularly mottled and calcified shadow in its interior and a fuzzy area of localized destruction of the cortex should make one suspect tumor of this type (Fig. 4). This suspicion is all the more justified if, where the cortex is undergoing destruction, it is somewhat thickened in part or throughout, and is overlaid by tissue casting an abnormal shadow.

In the absence of mottling and calcification as a clue to the cartilaginous nature of a central bone lesion, it will not be clear at all that a given malignant tumor in a long bone is a chondrosarcoma (Fig. 9). For instance, in one of our cases, when first seen, the lesion presented itself as a somewhat trabeculated but not particularly radiolucent area involving the lower quarter of the shaft (which was slightly expanded), and part of the external condyle of a femur. Were it not for the fact that, in the affected area, the cortex, especially anteriorly, appeared fuzzy, apparently in consequence of invasion by tissue from the medullary cavity, one would have had good reason to interpret the lesion at this stage as benign and possibly as a large solitary focus of fibrous dysplasia.²⁰ Within a few months there could be no doubt that the lesion under consideration was a malignant tumor, for by that time the roentgenogram (Fig. 10) showed extensive destruction of the cortex and the presence of a large, extra-osseous tumor mass. Still, neither in this mass nor in the tumor within the bone were there mottled opacities suggesting a cartilage growth undergoing calcification and

ossification. Altogether, in this case, if one did arrive at a diagnosis of chondrosarcoma roentgenographically, this conclusion could have been reached only by the process of elimination.

The peripheral chondrosarcomas are, on the whole, not difficult to single out. A benign osteochondroma, whether small or large, presents roentgenographically a more or less uniform texture and a well defined peripheral outline, beyond which there are no abnormal shadows. In contrast, an osteochondroma which has undergone transformation into a chondrosarcoma presents a dense, blotchy appearance over a considerable area, usually associated with the presence of more ragged, irregular radiopaque streaks extending away from the main part of the lesion (Figs. 13 and 14).

Treatment

The only form of therapy which offers any prospect of cure in cases of chondrosarcoma is surgery. Irradiation therapy is hardly of any value, since this type of tumor is highly resistant to such treatment, tending to continue or resume its growth in spite of it. Irradiation may serve at most as a palliative agent for a chondrosarcoma in a site inaccessible to surgical intervention, and should not be used with any higher expectation.

Surgical treatment of chondrosarcoma should be definitely on the radical side, and the wider the margin of supposedly normal tissue the better. A radical procedure offers the best promise of success when it is undertaken at the initial intervention. For instance, if the tumor seems, clinically and roentgenographically, to be confined to the lower half of a femur, but the surgeon, on amputating, finds cartilage in the marrow cavity of the bone at a level which he had thought would clear the lesion, it will be advisable to disarticulate at the hip joint immediately, also removing as much of the capsule of the hip joint and overlying muscle and other soft tissues as is feasible. Otherwise, a recurrence in the amputation stump is almost inevitable, and when this has taken place disarticulation at the hip joint is very likely to be too late to save the patient. As a part of the general principle of giving the lesion a wide margin in any site, the policy of keeping one joint ahead of the growth is a valuable one. Indeed, in connection with chondrosarcoma of one of the bones of the foot, for instance, it is advisable to amputate above the ankle joint rather than to attempt excision. On the same basis, if the lesion is in a rib, certainly several inches of the rib on each side beyond the region of apparent involvement, and at least corresponding sections of the rib above and below the affected one, should be sacrificed. The hopeful aspect of radical

surgery (and specifically of the prevention of recurrence by keeping well ahead of the growth) in cases of chondrosarcoma lies in the fact that as a rule the tumor has not yet metastasized at the time of the initial surgical intervention.

SUMMARY AND CONCLUSIONS

Chondrosarcoma is to be regarded as a lesion distinct from osteogenic sarcoma of bone. The former develops out of full-fledged cartilage, while the latter issues from more primitive tissue, developing out of bone-forming mesenchyme. Some chondrosarcomas do show large areas in which the intercellular matrix of the tumor cartilage has become heavily calcified or ossified, and in some osteogenic sarcomas cartilage in considerable amounts may be formed in the course of osteogenesis from the primitive mesenchyme. However, in a chondrosarcoma, in contrast to an osteogenic sarcoma, one never sees tumorous osteoid tissue and bone which is evolving out of a sarcomatous stroma directly, such as one always sees somewhere in an osteogenic sarcoma, no matter how much cartilage it contains.

In comparison with osteogenic sarcoma, chondrosarcoma is definitely less common, appears at a later age (on the average), runs a much slower course, and, especially if given radical surgical treatment at an early stage, has a much better prognosis, since the tumor has usually not yet metastasized at the time of initial surgical intervention. Even when the tumor is inadequately extirpated, it tends to recur only locally one or more times before extending to the tributary veins or to the lungs. Local trauma does not seem to be a factor in the initiation of chondrosarcoma or in the malignant transformation of the benign growths (enchondroma and osteochondroma) from which chondrosarcomas so often evolve.

A chondrosarcoma which begins its development within the interior of a bone may be denoted as a central chondrosarcoma, and one which begins in the cartilaginous cap of an osteochondroma as a peripheral chondrosarcoma. It is in the peripheral chondrosarcomas and those central ones which have clearly evolved from benign enchondromas that one finds, at least in the earlier stages of evolution of the lesion, heavy calcification or ossification of large parts of the intercellular matrix of the tumor cartilage. In other chondrosarcomas the relevant neoplastic tissue is likely to consist, in the main, of compacted islands of cartilage with hyaline matrix, though if the chondrosarcoma is very bulky one may also see areas in which the cartilage is softer and myxomatous, and perhaps even necrotic.

Although it is true that the histologic picture of a particular lesion

(irrespective of its gross appearance) does not have to be crudely and obviously sarcomatous to indicate chondrosarcoma, yet it is a mistake to suppose that to make a diagnosis of chondrosarcoma on a histologic basis alone is often difficult if not impossible. We think we have made it sufficiently clear that, even in the early stages of the evolution of a chondrosarcoma, one will find, at least in scattered fields, if adequate material is examined, subtle but tell-tale evidences of cytologic atypism of the cartilage cells which will betray the malignant character of the lesion. We hold that a cartilage tumor should no longer be regarded as benign if, when viable and not heavily calcified areas are examined, it shows, even in scattered fields: (1) many cells with plump nuclei; (2) more than an occasional cell with two such nuclei, and especially (3) any giant cartilage cells with large single or multiple nuclei or with clumps of chromatin. We feel that, by observing these criteria, the prevalent tendency to "underdiagnosis" of chondrosarcoma in an early stage of malignancy can be overcome. In a more fully evolved chondrosarcoma these indications will be relatively easy to find if one recognizes their diagnostic importance, and in a fully developed chondrosarcoma, of course, the histologic picture may even be obviously sarcomatous.

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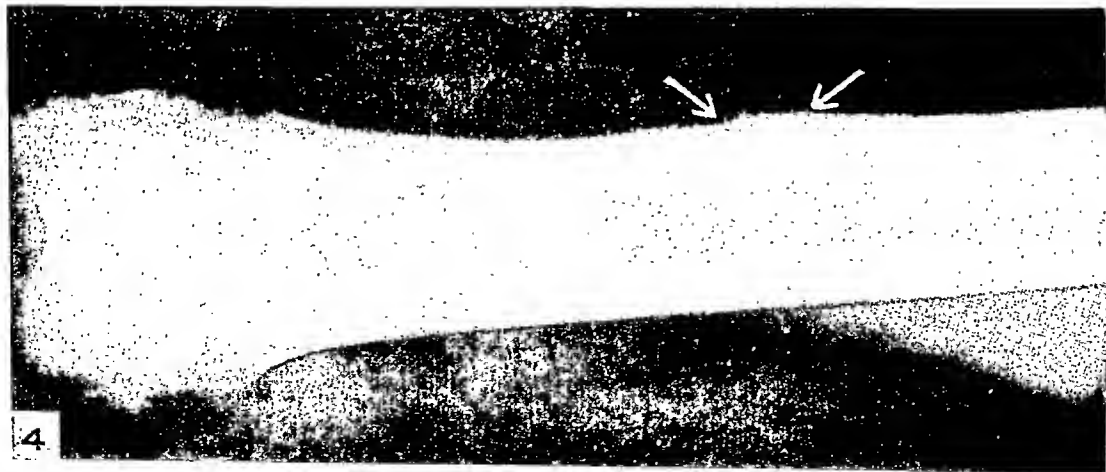
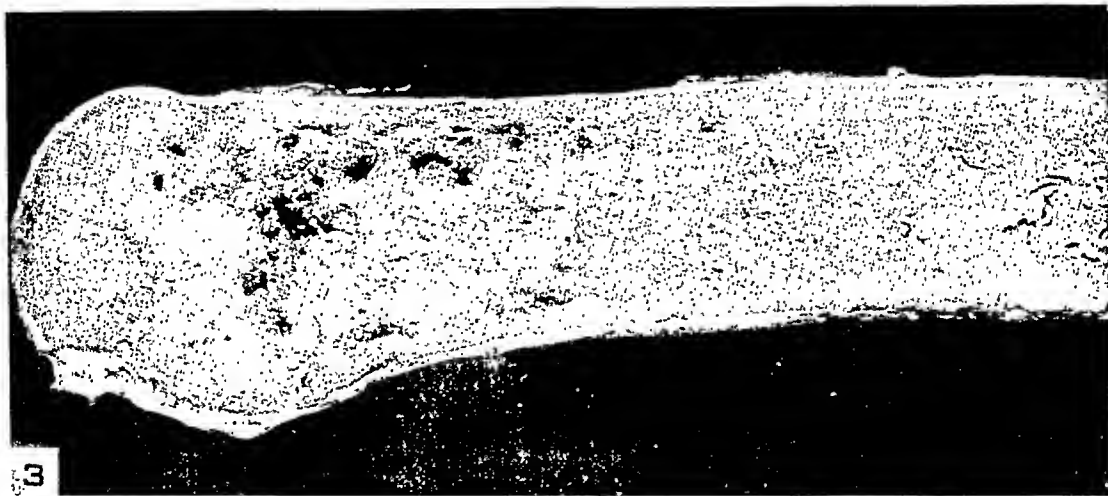
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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 63

- FIG. 1. Photograph showing sagittally cut surface of an upper end of a humerus presenting a chondrosarcoma early in the course of its evolution from a calcified and ossified enchondroma. It can be seen that the surgical and anatomic neck of the bone is somewhat expanded, and there are also indications that the articular surface is finely ridged and knobbed. Small foci of cartilage can be seen in the central part of the affected area, though on the whole this part is osseous. Peripherally, a cuff of hyaline cartilage of irregular thickness can be noted, and it was certain areas of this cuff that revealed the sarcomatous character of the lesion histologically. The periosteum has not yet been perforated at any point. (See Fig. 2 for roentgenographic, and Figs. 19 and 20 for cytologic features of this case.)
- FIG. 2. Roentgenograph of the affected portion of the humerus shown in Figure 1. The relative radiolucency at the periphery reflects the peripheral cuff of hyaline cartilage. The relative radiopacity and stippled appearance of the central part reflects calcification and ossification apparently dating back to the benign enchondromatous stage of the lesion.
- FIG. 3. Photograph showing sagittally cut surface of a portion of a femur with a chondrosarcoma which likewise appears to have evolved from a calcified and ossified benign enchondroma. The ablated portion of the femur showed no alteration in contour, except for the presence of a small tumescence antero-laterally (not demonstrated in the present photograph) at about the junction of the lower and middle thirds. The sectioned specimen revealed the presence, in the medullary cavity, of areas of white, glistening, and clearly cartilaginous tissue, alternating with areas of heavily calcified cartilage, along with a few areas which seemed to represent foci of ossification developing in such cartilage. By appropriate transverse sectioning of that half of the specimen which showed the mass mentioned above, it was found that in the area in question the cortex had been perforated by cartilage growing through at several points from the medullary cavity. (See Fig. 4 for roentgenographic, and Figs. 23 and 24 for cytologic features of this case.)
- FIG. 4. Roentgenograph (preoperative) of femur from the case illustrated in Figure 3. There is a radiopaque mottled shadow filling the interior of the bone and reflecting the presence of considerable calcification and ossification of the tumor. There is no alteration in the contour of the bone and no thickening of the cortex, except where the latter has been perforated (see arrows).



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Chondrosarcoma of Bone

PLATE 64

FIG. 5. The coronally cut surface of the lower end of an ulna with chondrosarcoma from a case of enchondromatosis involving also the homolateral radius and many of the bones of the corresponding hand. The ulnar lesion, as well as the lesions in the fourth finger, had shown a recent pronounced exacerbation of growth and had transgressed the original boundaries of the bones. The affected ulnar area and finger had therefore been resected. In the ulna may be seen compacted facets of hyaline cartilage composing the main mass of the tumor and continuous with the cartilage of the interior of the bone shaft. The scattered dark spots are foci of ossification, many of which are distributed through the entire lesion and which show up en masse in the roentgenograph of this specimen (Fig. 6). That this lesion was a chondrosarcoma was clear histologically, but there has been no recurrence during the 2 years since resection was done. Lesions such as this one in the ulna, in cases of enchondromatosis, are often misdiagnosed, at least roentgenographically, as osteochondromata. It is for this reason that one finds statements in the literature to the effect that, in some cases of multiple enchondromatosis, multiple osteochondromata are also present.

FIG. 6. Roentgenograph of specimen illustrated in Figure 5.

FIG. 7. The sagittally cut surface of a femur presenting a chondrosarcoma. The neoplastic tissue, though clearly present within the major marrow cavity and the marrow spaces of the spongiosa of the affected portion of the femur, has broken out of the bone without distending it and grown mainly in the surrounding soft parts. The neoplastic tissue appears whitish and glistening and was composed of smaller or larger facets of cartilage, separated by thin connective tissue septa. The tumor infiltrates a good part of the capsule of the knee joint, especially posteriorly. The large arteries and veins behind the knee were compressed, but no neoplastic tissue was found adherent to the walls of the veins. The patient in this case died at another hospital 7 years after the amputation, but no autopsy was secured. (See Fig. 17 for cytologic features.)



PLATE 65

FIG. 8. The sagittally cut surface of a portion of a femur presenting a chondrosarcoma. There is extensive destruction of the affected area of the bone, and bulging of the tumor mass anteriorly and into the joint cavity. A neoplastic fracture is obvious somewhat above the femoral condyles. The sectioned specimen revealed, over most of its surface, soft, glistening, whitish cartilage which in some places had an almost myxoid consistency while in others it was more or less divided into facets by septa. Hemorrhagically discolored areas were also present, as were areas which appeared necrotic and softened, apparently in consequence of the heavy irradiation therapy which had been given without influencing the continued growth of the lesion over a period of about 8 months. Nowhere was the neoplastic tissue calcified and ossified. The large veins draining the affected area did not show any tumor thrombi. (See Figs. 9 and 10 for roentgenographic, and Fig. 18 for cytologic features.)

FIG. 9. Initial roentgenograph, taken $8\frac{1}{2}$ months before amputation, of the lesion illustrated in Figure 8. On the basis of this roentgenograph, the lesion had been interpreted clinically as a giant cell tumor. The chondrosarcoma appears as a trabeculated rarefaction shadow and the cortex anteriorly is fuzzy and obviously in process of destruction. If there had been tell-tale spots of radiopacity reflecting calcification and ossification, the lesion could have been identified, clinically at least, as some sort of central cartilaginous growth.

FIG. 10. Roentgenograph taken $7\frac{1}{2}$ months after that shown in Figure 9, and 1 month before amputation. The progression of bone destruction in spite of irradiation therapy is apparent. The extensiveness of the lesion beyond the limits of the bone does not stand out, because of the absence of calcification and ossification in the neoplastic cartilage.



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PLATE 66

FIG. 11. Roentgenograph of both halves of the sternal end of a rib sectioned in its long axis, presenting a chondrosarcoma which had developed from an osteochondroma. The more radiolucent part represents the wide cartilage cap of the lesion, while the deeply radiopaque area represents the heavily calcified and ossified portion. (See Fig. 12 for the gross picture.)

FIG. 12. The cut surface of the upper half of the lesion shown roentgenographically in Figure 11. The photograph was made after the specimen had been in a fixative for years but one can still see, near the middle of the specimen, a white and gray speckled core representing the gritty, calcified and ossified portion of the lesion. To the right, this was capped by a wide zone of hyaline cartilage, which, at its widest, was as much as 1.5 cm. thick. (See Figs. 21 and 22 for cytologic features.)

FIG. 13. Roentgenograph of a chondrosarcoma springing from a sixth rib in a case of multiple exostoses. The shadow cast by the tumor is mottled but, on the whole, radiopaque, reflecting extensive calcification and ossification in it: In this picture, important details relating to the external periphery of the tumor are lost because in this reproduction the lesion appears only about one-third as large as in the original roentgenograph. In the latter, it could be clearly seen that at the external periphery of the lesion the areas of radiopacity fade out into a wide zone of radiolucent tissue representing tumor cartilage which has not yet become calcified. Such a picture is characteristic of a chondrosarcoma developing from an osteochondroma. (See Fig. 25 for cytologic features.)

FIG. 14. Roentgenograph (anteroposterior and oblique views) of a chondrosarcoma evolving from an osteochondroma at the upper end of a tibia. The areas of radiopacity indicate calcification and ossification in the tumor. In the upper part of the oblique view there are ragged, irregular radiopaque streaks extending from the main part of the lesion, which are due to strands of calcification and ossification in the tumor cartilage.



11



13



12



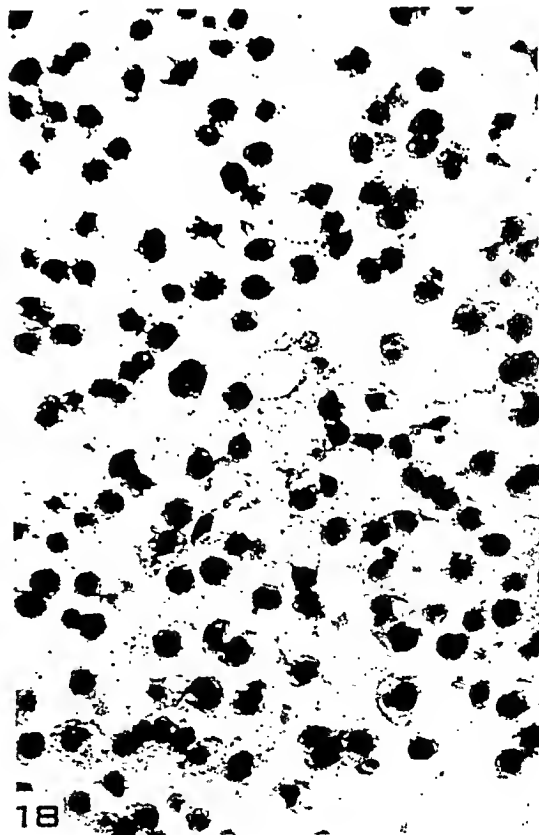
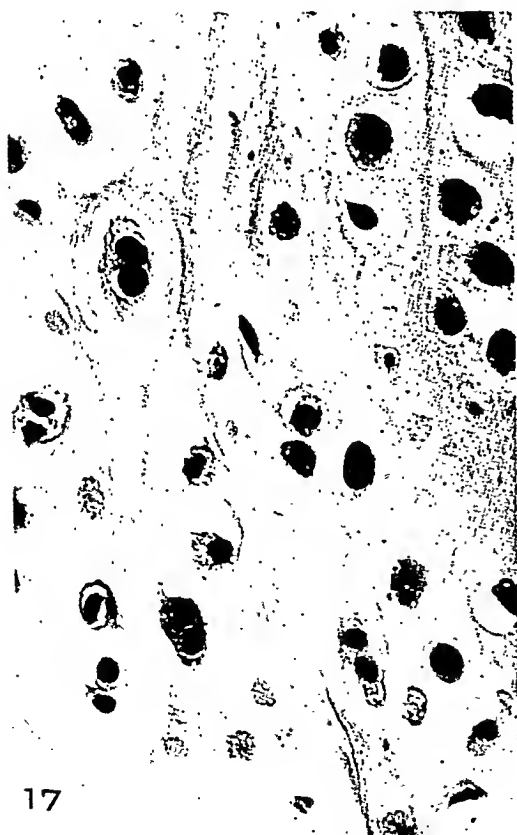
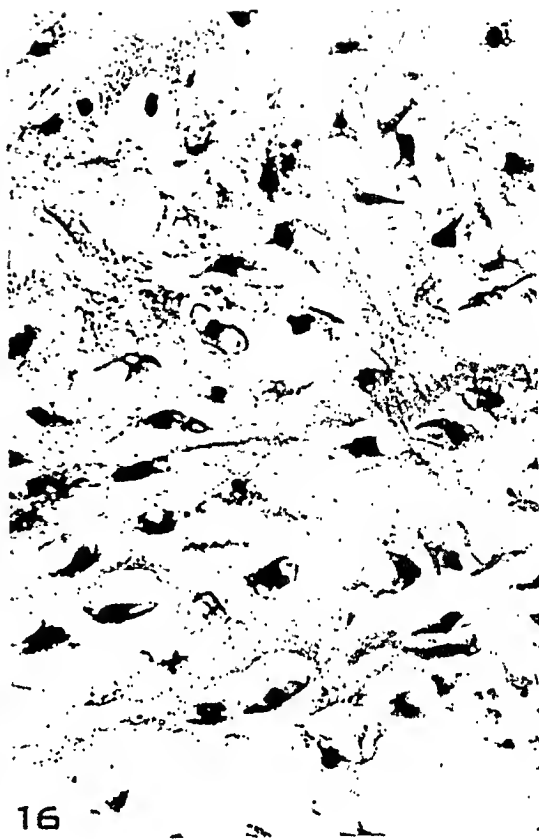
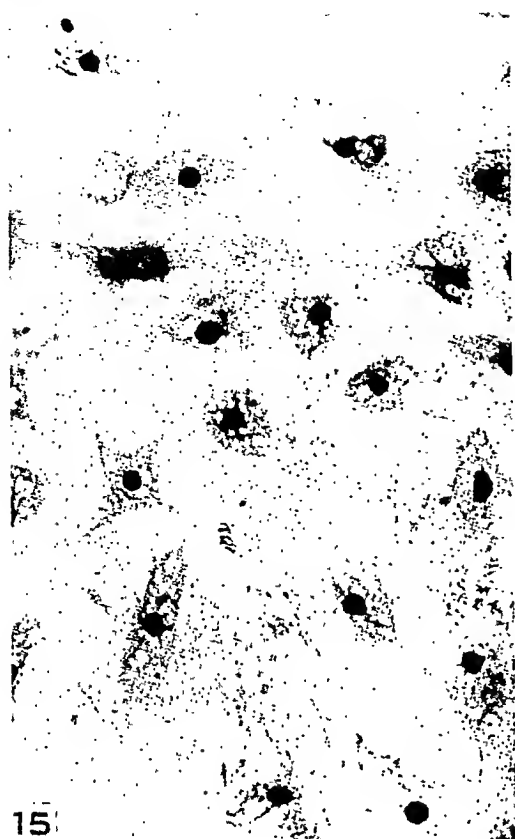
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PLATE 67

- FIG. 15. The histologic pattern of a typical field from a benign enchondroma. There are not very many cells in the field. The hazy and blurry appearance of the intercellular material is due to the presence of calcareous dust in it. Indeed, it is difficult to find, in a benign enchondroma, a field in which the intercellular material does not contain calcium, at least in the form of such tiny particles. The nuclei, though prominent, are small, especially in relation to the size of the cell as a whole. No cells with double nuclei are seen. $\times 250$.
- FIG. 16. The histologic pattern of a typical field from another benign enchondroma. Here the calcareous dust particles in the interstitial material are more abundant. Many of the cells have retracted in their lacunae, and in many both nucleus and cytoplasm appear drawn out. Again there are clearly no cells with double nuclei. $\times 250$.
- FIG. 17. The histologic pattern of a field typical of the viable tumor areas in the amputated specimen of the chondrosarcoma shown in Figure 7. At least five cells with double nuclei can be seen. On the whole, the nuclei here are much more prominent than those in Figures 15 and 16. $\times 250$.
- FIG. 18. The histologic pattern of a field typical of the viable tumor areas in the amputated specimen of the chondrosarcoma illustrated in Figure 8. The high cellularity of this tumor field is evident, and the nuclei tend to fill out the cells. Cells having two nuclei are numerous. $\times 250$.



Lichtenstein and Jaffe

Chondrosarcoma of Bone

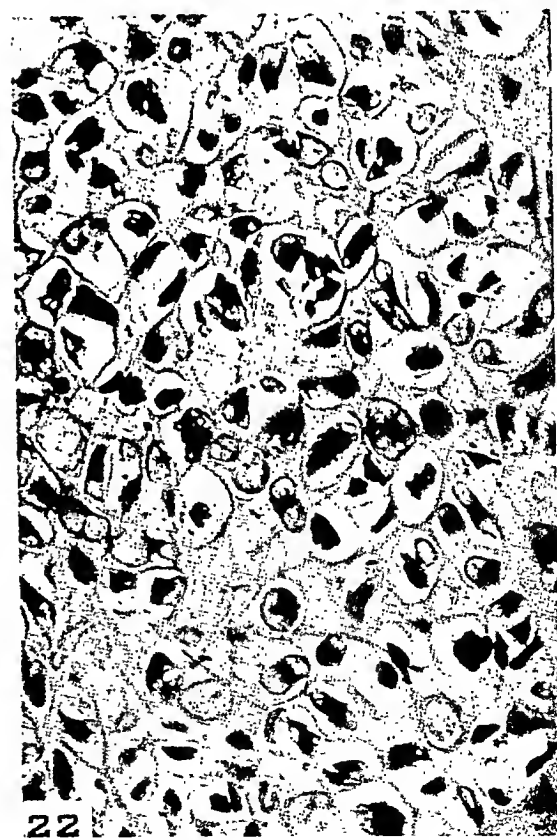
PLATE 68

FIG. 19. The histologic pattern of a typical field from the original specimen obtained for biopsy 10 months before the tumor illustrated in Figures 1 and 2 had been resected. On the basis of the histologic findings at that time, as shown here, the lesion was regarded as a benign enchondroma. The cells are relatively few, and lie retracted in lacunae; the nuclei are relatively small, and apparently only one cell is binuclear. $\times 250$.

FIG. 20. The histologic pattern in scattered fields of the resected specimen illustrated in Figures 1 and 2. (Compare also with Fig. 19.) Now, 10 months after the original biopsy, the ominous fields are relatively more cellular and show many cells with two and even some with four nuclei. On the basis of even scattered fields like this, the lesion can be regarded as definitely a sarcoma. $\times 250$.

FIG. 21. The histologic pattern of some fields in the cartilage cap of the lesion illustrated in Figures 11 and 12. On the basis of such fields, and particularly because of the presence of relatively many cells with two nuclei, the lesion, even at this stage, must be interpreted as a chondrosarcoma and a recurrence is certainly to be expected unless the lesion is very widely excised. The lesion did recur (see Fig. 22). $\times 250$.

FIG. 22. The histologic pattern in the recurrence of the chondrosarcoma illustrated in Figure 21. There is now a great increase in cellularity, cells with double nuclei are numerous, and there are many cells with very large single nuclei. $\times 250$.



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PLATE 69

FIG. 23. The histologic pattern typical for fields in the area of perforation and for scattered fields in the cartilage within the medullary cavity of the amputated portion of the femur and in the curettings from the femoral stump in the case illustrated in Figure 3. On the basis of such fields, the lesion must be regarded as a chondrosarcoma and recurrence expected, since cartilage of this character had remained in the femoral stump. (See Fig. 24 for the histologic character of the recurrence.) $\times 250$.

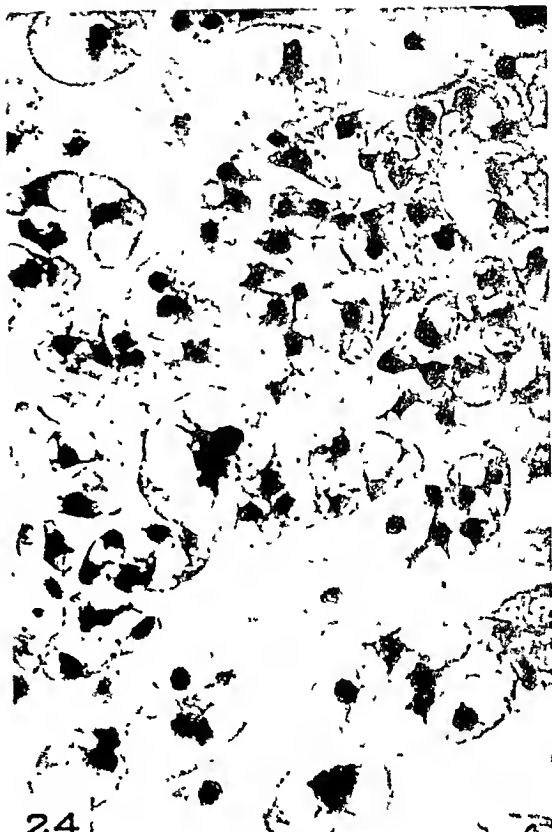
FIG. 24. The histologic pattern, typical for all viable areas, of the recurrence in the femoral stump of the chondrosarcoma illustrated in Figure 3. One finds now pronounced cellularity of the tumor cartilage and numerous binuclear cells. $\times 250$.

FIG. 25. The histologic pattern, for all viable areas, of the large chondrosarcomatous tumor mass encasing the lung and found at autopsy in the case illustrated in Figure 13. The original neoplastic tissue obtained at resection of the affected rib in this case presented, on the whole, a cytologic character like that shown in the lower half of the present figure. Even at this relatively low magnification, many binuclear cells can be seen in this half of the picture. In the upper half, many giant cartilage cells with large nuclei are to be seen. Altogether, this picture is one of a fully developed chondrosarcoma. $\times 100$.

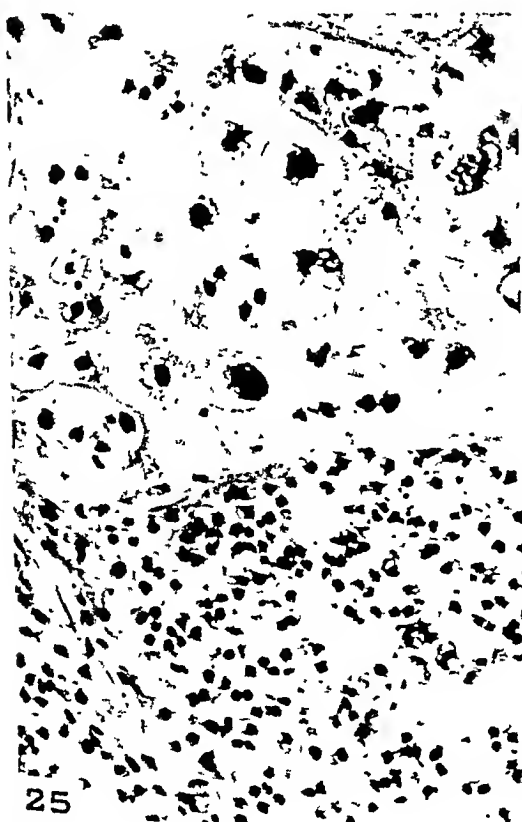
FIG. 26. The histologic pattern of a tumor composed of collagenous spindle-celled connective tissue which is undergoing myxomatous degeneration. At first glance, and uncritically, one might regard this tissue as from a chondrosarcoma. $\times 100$.



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TUMORS OF SWEAT GLANDS *

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A. HYPERTROPHY, HYPERPLASIA AND METAPLASIA

The sweat gland, unlike the sebaceous gland, seldom shows hypertrophy or hyperplasia as a primary change, but does do so as a process secondary to such lesions as nevi, angiomas, or hydradenoma ‡ papilliferum.¹⁻³ In some instances hyperplasia may suggest neoplasia. In Figure 2, there is shown hyperplasia of the secretory portion of the gland. This represents one of several hyperplastic glands which formed a tumor-like enlargement on the abdominal wall of a middle-aged woman. Cystic dilatation combined with the hyperplasia in some glands made the resemblance to neoplasia even more striking.

An unusual multiplication of glands is shown in Figure 1. The specimen came from the occipital region of a child, 5 years old, and had been present since birth.

A remarkable case was reported by Stokes⁴ of a girl, 19 years old, who had marked enlargement and pigmentation of her leg due to a diffuse congenital angioma and a hyperplasia of the sweat glands associated with hyperhydrosis. The angioma responded to x-ray treatment but the sweat glands were unaffected.

Epithelial metaplasia, commonly associated with chronic irritation, is only occasionally seen in sweat glands although they are frequently irritated or inflamed. This may be related to the constant drainage of the glands.

We have noted single apocrine-like structures among coil glands, associated with chronic inflammation, on parts of the body where apocrine glands do not usually occur. It is a question whether this represents changed epithelium of sweat glands under abnormal conditions and, if so, whether it is properly considered a metaplastic change. In Figure 3 we present an example of metaplasia of the secretory alveoli in an inflammatory lesion of the hand.

Walther and Montgomery⁵ reported metaplasia of the ducts in a palm of a woman, 29 years old, associated with nonspecific dermatitis and deep discharging fissures, present since birth. The metaplastic

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‡ The spelling "hydradenoma" is sanctioned by general usage. However, the correct form is "hidradenoma" since the derivation should be considered as from ἵδρω̃ς (sweat) and not from ὕδωρ (water).

dilated ducts showed both mucus and keratin; the secreting portions of the glands were normal.

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B. TUMORS OF TRUE SWEAT GLANDS

The approach to tumors of sweat glands has been scholastic for so long that a consideration of the literature forms a necessary part of a report on the subject. This is illustrated by the introduction to the section on sweat glands in a recognized textbook:⁶¹ "The reader is earnestly advised to refer to the literature given at the end of this chapter in order to acquaint himself in detail with this difficult part of dermatological histology." To this we demur. The literature is confusing and largely casuistic but we feel that more critical observation of the tumors will bring out only those difficulties attendant on the interpretation of any neoplastic process. Borderlines between hyperplasia, benign tumor, or malignant tumor are always problematical and questions of etiology may be as yet unanswerable.

In the early literature there were three main controversial points in differentiation of tumors of sweat glands: (1) distinction from tumors of other structures; (2) differentiation from hyperplasia of the glands; (3) recognition of tumors developing from the secretory and from the ductal portions of the gland.

The tumors of sweat glands were at first confused with endotheliomas and angiomas, as were all the nonkeratinized epitheliomas of the skin. Their peculiar tubular structure and the multiplicity of lesions set them apart as "hemangio-endothelioma tuberosum multiplex"⁵⁰ and "lymphangioma tuberosum multiplex."⁵² Lotzbeck⁵⁸ in 1859 reported a tumor that he believed to be of sweat gland origin. Unna⁹⁵ accepted the author's interpretation, although he stated that Virchow considered the tumor angiomatous. On the other hand, some tumors that Unna attributed to sweat glands seem to us doubtful and might well pass for glomus tumors.^{11, 47}

The differentiation between hyperplasia and neoplasia received much attention from the first observers of changes in sweat glands.^{90, 95} The adenoma sudoriparum described by Verneuil⁹⁶ and other French writers was criticized by Unna⁹⁵ as including nonneoplastic changes. The many varieties of tumor-like proliferation of the glands described in the literature all show loss of normal architecture in a greater or less degree. A slight epithelial proliferation of sweat glands is not unusual, but increase in the number of glands as well is not commonly recognized. We have seen only one example of proliferation of glands in which the alveolar structure closely resembled the normal gland, while the diffuse nature of the growth and cystic dilatation suggested neoplasia.

The early classifications were concerned especially with the part of the gland involved in the neoplastic process—the secretory alveolus or duct. These two parts of the gland are distinguished by the appearance and arrangement of the epithelial cells and by the myoepithelium around the secretory alveoli. The differences in the epithelium are so slight as to be readily lost with abnormal proliferation. The myoepithelium, normally quite easily seen, is rarely recognized in tumors.^{52, 60, 84} Both Buxton⁷ and Unna⁹⁵ gave careful descriptions of tumors arising from the two parts of the gland. Unna considered them distinct on the basis of etiology. The spiradenoma,^{2, 90} or tumor of secretory alveoli, he described as secondary to other lesions of the skin such as carcinoma, angioma, or varices, while the tumor of ducts, the syringadenoma, he interpreted as being congenital since it often appeared early in life and was not obviously associated with other conditions. Nevertheless, he granted the almost complete similarity of appearance except for hyaline or horny material present in cysts of the syringadenoma. The distinction between the spiradenoma and syringadenoma is now seldom made.

The origin of the tumors was stressed in the early classifications.^{74, 86} But the factors on which the decisions were based, the relation of the tumor with other structures of the skin, the presence of secretion within the tumor, the status of adjacent sweat glands, their presence, absence, or abnormality, led to a diversity of interpretations.

In spite of a number of case reports and several more or less complete reviews in the past 40 years,^{57, 77} the tumors are still ill-defined. The terminology of the early reports is only slightly modified today, and carries some of the uncertainty of the first observers. This is in part due to the comparative rarity and benignity of the tumors and to the absence of distinguishing clinical features.^{16, 69} The tumors are slowly growing but may become very large. They are either mul-

multiple or solitary and may appear at any time of life, although a great majority arise in youth or on maturity. Some types are clearly congenital. Ulceration is unusual. They have been described on every part of the body, but some forms are common on the chest, others on the vulva, and others on the upper face around the eyes and scalp. The hands and feet are not unusual sites. An accurate clinical diagnosis is difficult with the exception of the types which have a typical distribution such as the syringoma of the chest and the hydradenoma of the vulva. Knowledge of the structure has been restricted because biopsy or treatment is seldom indicated.

We propose to correlate our experience with the literature. We recognize four types of tumors of sweat glands having distinctive patterns: (1) syringoma; (2) hydradenoma papilliferum; (3) hydradenoma; (4) hydradenoid carcinoma. Our terminology has been chosen with regard to histologic suitability and historical and clinical usage. We will give a brief gross and a rather more detailed histologic description, a discussion of the synonymy and an illustrative photomicrograph for each group.

1. *Syringoma*

This tumor is typically small, under 1 cm. in diameter, and multiple; but an occasional solitary tumor may be three or four times as large. It is slightly elevated, smooth, semitranslucent and colorless or slightly discolored. This condition affects females especially at puberty but it may appear first in middle life. There is some disagreement as to the usual distribution of the tumors, because of their close resemblance to epithelioma adenoides cysticum * (except for a less yellow color),⁹⁵ but it is probable that the latter is more often found on the center of the face and lower eyelids and the syringoma on the chest and axilla. Feit and Kelley³⁰ described a case with lesions distributed on the lower torso and thighs. Ullmo⁹⁴ reported a very curious case: a woman of 35 years, on becoming pregnant, developed hundreds of small, rounded, pinkish papules over the skin of the chest, neck, axillae and breast. There were no illustrations, but the descriptions correspond with our syringoma. These tumors are radiosensitive.^{37, 45, 67}

The microscopic structure consists of epithelial groups scattered haphazardly through the corium usually without appreciable disorganization of the general architecture of the dermis. The separate unit

* A discussion of epithelioma adenoides cysticum may be found in our paper on epithelial cysts and cystic tumors of the skin to be published in the September issue of the Journal.

is a fairly short, narrow, sometimes branching strand of epithelial cells about the diameter of a normal coil duct, which it closely resembles. Cystic expansion, apparently the result of degeneration, adds to the similarity. The absence of myoepithelium⁶⁰ suggests origin from the ducts rather than the coils. The cysts are lined by cuboidal or flattened cells and contain clear fluid or hyaline or, rarely, horny material. Some authors have assumed that the fluid is the product of secretion. There may be a distinct basement membrane.

Civatte and Chevallier¹⁴ described a lesion of unusual type, possibly belonging to this group. A number of subcutaneous lesions on the breast of a woman, 19 years old, were formed of discrete collections of sweat glands and dense fibrous tissue in the nature of a fibroadenoma. The report was not illustrated and it is difficult to be sure of their exact nature.

The sweat glands adjacent to the tumor may be normal, absent, or abnormal. Török⁹² and Unna⁹⁵ stressed the absence of normal sweat glands in the vicinity of the tumor as evidence bearing on their congenital origin. Petersen,⁷² in 1893, finally concluded that a tumor which he had previously reported⁷³ in 1892 as congenital, was from fully developed sweat glands. Edel²⁵ demonstrated syringoma developing from apparently normal ducts. The most commonly held view is that the tumor originates from embryonic sweat glands^{16, 75, 101} and either grows slowly or not at all until puberty or later. A few writers have regarded these tumors as nevi of apocrine gland origin.¹⁰⁰

We have only one tumor that may possibly belong to this class, although the clinical features were unusual. It came from the scalp of a male, 26 years old. It measured 0.8 cm. in diameter and had been noticed for only 3 months. The histologic structure was quite typical, however, except for a nodular grouping of the individual epithelial units and a rather prominent mucinous change in the stroma.

The tumor is known by several different names:

1. Syringoma or syringadenoma (Dohi,²⁰ Eller,²⁷ Gans,³⁹ Unna⁹⁵)
2. Syringocystadenoma (McCarthy,⁶¹ Sutton and Sutton,⁸⁸ Török⁹²)
3. Syringocystoma (Finnerud,³³ White¹⁰³)
4. Hydradénome éruptif (Jacquet and Darier⁴⁹)
5. Cellules epitheliales éruptifs (Quinquaud⁷⁵)
6. Lymphangioma tuberosum multiplex (Kaposi⁵²)
7. Hemangio-endothelioma tuberosum multiplex (Jarisch⁵⁰)
8. Spiradenoma (Ewing²⁹)
9. Naevi cystepitheliomatosi disseminati (Pernet⁷¹)

2. *Hydradenoma Papilliferum*

Hydradenoma papilliferum is a papillary growth of quite variable structure. It is particularly common on the labia, perineum and thighs,^{4, 6, 26, 46, 62, 89, 108} but also occurs on the face, especially the upper lip, and on the scalp. It is more apt to be solitary than multiple, usually is noticed in middle life and grows slowly, but is generally not more than 2 cm. in diameter. The histologic structure is essentially papillary, expansile and discrete, but not encapsulated, and varies according to the complexity of the papillary ramifications, the proportion of solid growth, the tendency to degeneration and the amount of stroma. The epithelium is two-layered, the cells bordering the lumen are high-columnar. The nucleus has a central or basal position and the cytoplasm is typically faintly bluish or colorless. But it is not unusual to find here and there groups of rather squat eosinophilic epithelial cells reminiscent of apocrine glands. The papillae may be narrow papillary folds projecting into cystic spaces or complex, branching, intracystic growths. In the nonpapillary parts, the cells may form a loose mesh of glandular spaces with almost no stroma or they are closely packed together in a patternless mass. These latter seem to be formed in some instances by proliferation of basal cells of the ducts. Mitotic figures are not particularly numerous and there is rarely any appreciable degree of anaplasia. Cysts are formed in two ways: by growth and dilatation of the gland, and by degeneration within the tumor. A marked degree of necrosis is unusual. In a few tumors with predominant stroma, the cysts are stretched into cleft-like spaces and the resemblance to adenofibroma of the breast is striking. The growth may be completely intracystic. Many of the tumors arise in the lower corium or subcutaneous tissue. Not infrequently the tumor extends through the whole thickness of the corium. Rarely the tumor arises very near the epidermis. It has been surmised that the tumor arises from the duct. The epithelium is not characteristic of either part of the gland and the absence of typical myoepithelium probably is not significant.

Very few of the early writers considered this type of tumor in a separate category. We assume that it has been classed with nevi of sweat glands,²⁷ i.e., either as naevus syringadenomatosus or as naevus syringocystadenoma papilliferus. Pick's⁷⁴ illustration (Fig. 6, p. 341), and Ruge's⁷⁹ illustration (p. 313), coincide with our hydradenoma papilliferum. Hoeck's⁴⁶ tumor of the vulva called tubular hydradenoma probably belongs to our deep hydradenoma papilliferum, although the photograph seems less typical than the description. Blau⁴ showed very good illustrations of the more solid type of hydradenoma papilliferum which he described as an adenoma consisting principally of tubules.

We believe that the majority of the tumors described as adenocarcinoma of the vulva⁶² are benign and should be considered hydradenoma papilliferum.

There are two less common forms of hydradenoma papilliferum which may conveniently be termed (a) superficial and (b) intermediate. The superficial hydradenoma papilliferum is a tumor of the terminal ducts, primarily. There is dilatation of the ducts, but usually not to cystic proportions, and proliferation of the epithelium forming low papillary infoldings. Intraductal growths are sometimes seen but they are not an essential part of the picture. Perhaps the most striking change is in the character of the epithelium. The two layers of cells of the normal duct usually remain distinct. The lining cells become tall-columnar. The cytoplasm is commonly faintly basophilic and the nucleus lies in the center or at the base of the cell. The free border of the cell may be lipped or frayed. Near the orifice there is frequently a pseudostratification of cells and occasionally squamous metaplasia and keratinization. Alterations of the secretory part of the gland consisting of dilatation and varying degrees of hyperplasia seem to be secondary to the changes in the terminal ducts, but occasionally the epithelial proliferation in the secretory alveoli is marked.⁵⁴ A moderate fibroblastic proliferation and inflammatory reaction in the surrounding corium usually appear as an integral part of the process.

The gross characteristics are not distinctive. The tumor may be large or small, more or less papillomatous or polypoid, or form a plaque by confluence of small growths. It sometimes resembles a condyloma. It has been described as occurring chiefly on the face,⁷⁶ on the scalp²⁷ and in the region of the shoulder, axillae, or genito-inguinal folds.³⁸ A pore-like opening is often present from which serosanguinous fluid and papillary structures may be expressed and it is frequently crusted with hyperkeratotic scales or dried secretion.

The nature of the process has not been definitely proved. Some of the tumors have been described as congenital.⁶¹ The association with other skin lesions as naevus unius lateris²⁸ and chronic eczema⁷⁶ and the inflammatory changes, has suggested a reactive process.⁷

We have seen only two typical examples of the superficial hydradenoma papilliferum (41-3362, 10316). A third tumor (37-2022), classified with the carcinomas may have been originally a superficial hydradenoma papilliferum. It was a congenital lesion in which malignancy became manifest in middle life. In the center of the tumor were enlarged ducts lined by proliferating epithelium with dilated and slightly hyperplastic secretory alveoli beneath. On each side of the tumor was typical basal cell carcinoma arising from the superficial epi-

thelium and from hair follicles. The carcinoma extended to a slight extent through the midcorium between the secretory and the ductal portions of the lesion. Fibrosis of the stroma was rather marked.

Reviews of the literature in 1936³ and in 1933⁷⁶ give a total of 33 published cases of the superficial hydradenoma papilliferum. It has been commonly known as naevus syringocystadenomatosus papilliferus.^{27, 31, 38, 76, 80} Elliott²⁸ described the tumor as adenocystoma intracanaliculare. Unna's⁹⁵ cylindrical-celled cancer (p. 711), which he described as arising from metaplasia of surface epithelium, may possibly conform to the superficial hydradenoma papilliferum. Werther's¹⁶² syringadenoma papilliferum (naevus syringoadenomatosus papilliferus) has some of the characteristics of our superficial type of hydradenoma papilliferum but it seems to us to have more in common with the usual form of hydradenoma papilliferum.

The intermediate type of hydradenoma is a rare tumor which suggests a growth from a congenital abnormality. There are no distinctive clinical characteristics. It is a tubular, branching structure lying usually in the upper or middle corium. Epithelial proliferation is sluggish and there is relatively little tendency to stratification of cells, papillary infoldings, or solid growth. It is generally classed with the naevi syringoadenomatosi and considered congenital. McCarthy's⁶¹ and Wolters'¹⁰⁷ description of naevus syringoadenomatosus and Eller's²⁷ sweat gland nevus show somewhat more proliferation than we have seen in the tumors in this group, but otherwise are similar.

The tumors we have classified as varieties of hydradenoma papilliferum have been described in the literature as distinct tumors. In thus grouping them we do not wish to imply any relationship other than a similarity of the type of growth. There are many differences. The superficial tumors are often associated with inflammation; both superficial and intermediate types have characteristics suggesting a congenital malformation, and the deep tumors are more complex neoplasms, probably acquired. Atypical forms are frequently described that do not fall clearly into any group. Many of these appear to be congenital.^{10, 63}

3. *Hydradenoma*

Hydradenoma is a solid tumor of sweat gland. This is more common than all the other tumors of sweat glands together, and less frequently recognized as originating from these structures. It is a grossly discrete, solitary or multiple growth, sometimes appearing in groups, that is found chiefly, although not exclusively, on the face and scalp. It produces a low mound-like elevation, except on the scalp where it may be pedunculated. The tumor is frequently located in the subcutaneous tissue and lower corium but may involve the entire corium. Rarely it

seems to arise close to the epidermis. Ulceration is unusual. The tumor may be any size but is rarely more than 4 cm. in diameter. Growth is slowly progressive to a certain limit and thereafter the tumor usually remains of the same size.

In a few tumors, a partly papillary structure suggests development from a hydradenoma papilliferum, but the majority consist essentially of alveolar spaces embedded in compactly arranged, solid cell masses and strands. The alveoli, numerous or only occasional, have distinct cuboidal or columnar epithelium-lining cells with central or basal nuclei and are cuffed by more or less massive proliferation of smaller cells characterized by hyperchromatic nuclei and inconspicuous cytoplasm. The appearance of the alveolar cells and of the solidly massed cells is sometimes quite similar except for shape. Frequently, however, the alveolar cells stand out in sharp contrast, either because of deeply eosinophilic cytoplasm or because of abundant rather pale cytoplasm. The resemblance of these pale lining cells to normal secretory epithelium suggests origin from it, but these cells may also be seen in abnormal proliferation of the epithelium of the ducts (Figs. 4 and 5). We have not attempted to distinguish between hydradenoma of ducts and of alveoli because of the fact that under abnormal conditions the epithelium of these structures may have a similar appearance.

Although the alveoli are an essential part of the tumor, the diagnosis does not depend upon their presence but can be made on the architecture of the tumor and on the cell type. The distinct cell type that forms the solid masses may be described most clearly by comparison with the basal cell. It is rounded or polygonal rather than spindle in shape, and somewhat larger than the basal cell. The centrally placed nucleus is of medium size compared to the cytoplasm. There is considerable chromatin, but the nuclei are usually less dense than those of basal cells. Nucleoli are not prominent. The cytoplasm is quite variable in amount; it may be negligible or conspicuous. Some of the nuclei are naked, and, at the other extreme, the clear ballooned cytoplasm may suggest sebaceous epithelium,⁴¹ except for its reticulated rather than foamy structure. Generally the cells have somewhat more cytoplasm than the basal cells and it is either faintly eosinophilic or basophilic, less commonly clear. The limiting membrane is sharp. Mitotic figures are not usually conspicuous. A few tumors show invasive growth and, rarely, anaplasia. Sutton⁸⁷ explained the hydradenoma, which he called syringocystadenoma nodularis, as a proliferation of sheath cells of the gland rather than as a proliferation of the secretory epithelium. This conjecture serves to emphasize the typical appearance.

In occasional tumors, this typical appearance is not seen and there

may be a close resemblance to basal cells or squamous cells. A few hydradenomas are continuous at one or more points with the epidermis and there is keratinization of the superficial part of the growth. Nevertheless, the manner of growth is usually more suggestive of metaplasia of duct epithelium than invasive growth of epidermis. Necrosis seems to be less common in relation to tumors of sweat glands than in other nonkeratinized epithelial tumors.

The architecture is a very helpful differential feature. The tumors lie in the mid- or lower corium and are not typically connected with any of the epithelial structures of the skin. The masses of cells are compactly arranged units in distinction from the loose, lobulated, or reticulated growth often seen in basal cell carcinomas. The alveoli are not to be confused with cystic spaces found in tumors of the hair matrix or basal cells as a result of degeneration of the stroma or of the tumor. They are less easily distinguished from the true alveoli of mucous gland tumors of the lip. The cells, though closely packed, are not uniformly aligned, and often have no clear orientation to one another. There is rarely any peripheral palisading as in tumors of basal cells or of the hair matrix.

In certain of the hydradenomas there is a characteristic tendency toward hyaline degeneration (Fig. 5). This appears to start as a hyaline or amyloid-like change either in the basement membrane, or as a fine ribbon weaving among the epithelial cells, and less commonly as a degenerative change within the cells. Finally, the major part of the tumor may be replaced by broad strands and masses of hyalin with squeezed epithelial cells in the interstices. This change has been described by Unna.⁹⁵ Although hyalin is found in the nonkeratinized tumors of basal cell or hair matrix origin, it is more clearly the result of degeneration of the tumor or stroma and the pattern of deposition is different.

There have been wide differences of opinion in respect to the nature of the hydradenomas. They have been considered as arising from hair follicles and sebaceous glands as well as from sweat glands^{51, 78, 82, 98} and endothelium.⁸⁵ Location on the scalp has led to their inclusion with the ill-defined group of turban tumors. It must be acknowledged that in those hydradenomas without alveoli, origin from sweat glands cannot be proved. Furthermore, in some of the tumors, connection can be traced with the overlying epidermis or with hair follicles. The structure also has a certain resemblance to hair matrix carcinoma: narrow branching strands of cells, scattered whorls of nuclei about minute hyaline particles, and rarely, well formed hair follicles embedded in the tumor, not as an accidental inclusion but as an integral part of the growth.

Thirteen of our tumors were from both sweat glands and hair follicles. In one of these there was a suggestion of growth from sebaceous glands, but as basal cells rather than as differentiated sebaceous cells. Stromal proliferation is especially prominent in these tumors of both sweat glands and hair follicles.

The hydradenoma has been generally called cylindroma. There are several objections to this term. According to some usage, it carries a specific reference to hyalin. This is found in Ewing's ²⁹ definition of cylindroma: "a tumor structure in which the stroma appears in the form of elongated, twisted, thickened cords of hyaline material." Certain tumors of salivary glands and nasal sinuses characterized by hyalin and cord-like arrangement of cells have been called cylindromas. A term so generally applicable leads to confusion of tumors of diverse origin.

Examples in the literature of our hydradenoma may be found in Krompecher's ⁵⁵ illustrations (Figs. 6, 8 and 11) under the name cylindroma; in Watanabe's ⁹⁸ description and plates of cylindroma; in Figure 5 of the sweat gland adenoma described by Weidman and Besancon; ⁹⁹ and in Figures 20-A and 20-B of Geschickter and Koehler's ⁴⁰ paper on epithelial tumors of the skin, and in many other papers on cylindroma and turban tumors.

4. *Hydradenoid Carcinoma*

Hydradenoid carcinoma is a carcinoma of sweat glands. Carcinoma of sweat glands has never been clearly defined in the literature and little care has been taken to differentiate it from other nonkeratinizing carcinomas on the one hand and from adenoma on the other. Moreover, the propriety of this classification of sweat gland tumors is questionable in view of the well known fact that they are not often clinically malignant. Malignancy has been attributed to each of the three types of benign tumor: syringoma, hydradenoma papilliferum, and hydradenoma. However, malignant degeneration of syringoma and the more differentiated types of hydradenoma papilliferum is probably very rare if it occurs at all. Certainly, some of those carcinomas described as arising from syringoma and hydradenoma papilliferum of the superficial type are more suggestive of coincident basal cell carcinoma.^{76, 104} (as in our case 37-2022). Adenocarcinoma of sweat glands is described not infrequently, and usually as arising in hydradenoma papilliferum, notably of the vulva. In our opinion this diagnosis is seldom justifiable. The tumors are considered malignant on the basis of marked cellularity and absence of capsule and in most cases without any appreciable loss of differentiation. The tumors are easily shelled out and usually do not recur or metastasize. The thirty cases of "adenocarcinoma" of the

vulva recently published by McDonald⁶² illustrate the problem. Ewing²⁹ mentioned adenocarcinoma of the sweat glands as a tumor which metastasizes late, but he did not give any criteria for distinguishing the adenocarcinoma from the adenoma. His illustrations entitled "tumor" and "adenocarcinoma" of sweat glands (Figs. 461 and 462, pages 906 and 907) correspond to our solid tumor or hydradenoma.

The commonest form of carcinoma is the solid carcinoma, and in most instances it results from malignant change of hydradenoma. Still, the differentiation of benign and malignant varieties of the hydradenoma has not been definitely established. Some observers consider all the solid tumors malignant. The uncertainty of diagnosis of the solid carcinomas is due in great part to theoretic consideration and to overemphasis of unusual tumors. It has been assumed that malignant tumors of sweat glands lose all their characteristic features on becoming malignant.^{17, 95} Others have considered basal cell carcinoma or rodent ulcer as originating from any of the epithelial structures of the skin, sebaceous glands, hair follicles, and basal epithelium.^{12, 19, 40, 55, 91, 97} Uchiki and Suzuki⁹³ spoke of a specific "medullary form" of basal cell carcinoma arising from the ducts. Unna⁹⁵ described a cylindrical cancer of sweat glands having a tubular structure with "widespread, central liquefaction." The cavity formation seems to have been a process of degeneration rather than of true alveolar formation. He suggested that the secretion of the cells might play a part in the degeneration. Dörffel²¹⁻²⁴ emphasized the basal cell origin of tumors of sweat glands and sebaceous glands, and cited a tumor of sweat gland that had sebaceous elements in the recurrence. Borrmann⁵ illustrated a "corium carcinoma" spreading beneath the skin and growing out of ducts of sweat glands and hair follicles. Nonkeratinizing carcinomas originating from two or more cutaneous structures are seen occasionally and it is quite possible that undifferentiated carcinomas of sweat glands may not be distinguished from basal cell carcinomas. However, carcinomas with perfectly clear structure of sweat glands have been described and our concern is with these. A few of the carcinomas with a high degree of histologic malignancy have metastasized and recurred.

In considering the clinical aspects, one must take into account the great lack of interest and diligence shown by most writers with respect to adequate "follow-up" of patients having any type of cutaneous cancer. Unfortunately most of our own cases were not followed an adequate length of time.

Hufschmitt and Diss's^{48,2} tumor recurred twice at short intervals. The recurrence of Woringer's¹⁰⁸ tumor took place after a 2-year interval. Eichenberg²⁶ reported an unusually interesting case of adenocarcinoma of the vulva with metastasis to inguinal lymph nodes. This was

believed to have developed from a *hydradenoma papilliferum*. There was marked proliferation with formation of solid masses and invasive growth. Other tumors that metastasized were reported by Moriconi⁶⁴ and Hedinger.⁴⁴

We have listed in Table I tumors described as carcinoma of sweat gland. We have not included all of the cases in the literature. Some were too vague or too unusual and others were not clearly tumors of sweat glands.^{34, 83, 84} These tabulated cases should not be considered as representative of carcinomas of sweat glands, as usually they have been reported because of features of peculiar interest. Pelagatti's⁷⁰ case, for example, suggests a basal cell carcinoma. Thierfelder⁹⁰ reported a tumor as *spiradenoma* which had nevertheless extended through the skull to meninges. Cornil's¹⁵ tumor was not accepted by Unna,⁹⁵ who considered it identical with his cylindrical type of cutaneous cancer, which we interpret as carcinoma of basal cells or of hair matrix. Fordyce's³⁵ tumor, described as adenocarcinoma, corresponds to our *hydradenoma*. In this tumor the alveoli were more numerous than is often the case and it was considered malignant on the basis of "invasive growth." Ruge's⁷⁹ case is interesting chiefly because of the amount of attention it has received in the literature. The tumor was essentially *hydradenoma papilliferum*, encapsulated except for a few places without basement membrane. Ruge, himself, was conservative, admitting that the case was not absolutely clear, but inclined toward the view of a low-grade carcinoma arising from an adenoma. Landsteiner⁵⁶ did not accept Ruge's case but Schiffmann⁸¹ did and reported a similarly doubtful tumor.

Several of the tumors present difficulties of interpretation,^{9, 42, 66, 68} because of atypical characteristics such as pearl formation.^{18, 81, 90} The high incidence of metastasis and recurrence among those tabulated is undoubtedly due to selection of the cases.

The long history in many cases suggests that the tumors arise from adenomas.^{36, 57} Christot¹³ reported a carcinoma interpreted as arising from sweat glands that had been present but quiescent since infancy but which had grown rapidly during the year and a half before removal and weighed 450 gm. He traced the development of the tumor from hypertrophy of the sweat glands through hyperplasia to epithelioma. The cancer of sweat gland described by Cornil¹⁵ has some similar points. A papillary tumor on the leg of a man, 59 years old, had been present since birth but had lately ulcerated. The author described hypertrophy of sweat glands and metamorphosis of the epithelium into the pavement type with large fat droplets.

In Table II are listed our tumors of sweat glands. Only six of our tumors were frankly malignant from the point of view of invasive

TABLE I
Reported Cases of Sweat Gland Carcinoma

Author	Date	Patient		Location	Size and appearance	Duration and symptoms	Treatment and results
		Age	Sex				
Cornill ¹⁵	1865	59	M	Leg	2 cm.; irregular	Entire life; ulcerated 6 months	Excised; no recurrence
Christott ¹³	1866	33	F	Back	Child's head; ulcerated	Since childhood; recent ulceration	
Thierfelder ⁹⁰	1870	50	F	Forehead at hair margin	Hen's egg	4 yrs.; invaded frontal bone to meninges	Operation; death from meningitis
Morison ¹⁶⁵	1887	30	F	Fifth toenail		5 yrs.; invaded tarsal bones	Excised; recurred once; died after 4 mos.
Darier ¹⁷	1889	71	M	Chin	Goose egg	2 mos.	
Wierzbowski ¹⁰⁵	1892			Rt. anterior thigh			
Fordyce ³⁵	1895	35	M	Leg	Ulcerated, firm; one-half of small egg		Excised
Campanini ⁹	1897	50	F	Forehead	Ulcerated; occurred in scar	5 yrs.	
Antonelli ¹	1902	43	F	Rt. labium majus	Multiple nodules	6 mos.	No metastasis
Deichstetter ¹⁸	1902	65	M	Lt. elbow laterally	8 x 11 cm.; ulcerated	1½ yr.	
Lusena ⁵⁹	1904	63	M	Gluteal region		Few mos.	
Ruge ⁷⁹	1905		F	Posterior commissure of vulva	0.7 x 1.1 cm.		
Wollheim ¹⁰⁶	1907		F	Cheek	Linseed		
Hedinger ¹¹	1911	48	F	Scalp	4 x 5 cm.; grayish red, ulcerated	Since childhood; growth 10 yrs. 6 yrs.	Excision of tumor and two lymph nodes, each 1 cm., with metastases
Ricker and Schwalb ⁷⁷	1914	45	F	Rt. upper eyelid	1.2 x 1.2 cm.		

TABLE I—(Continued)

Author	Date	Patient		Location	Size and appearance	Duration and symptoms	Treatment and results
		Age	Sex				
Schiffmann ⁸¹ Ohno ⁶⁶	1920 1924	70	F M	Labium majus Scalp	Bean; reddish 3 x 3 cm.; eroded, red, hard margins, ulcerated	2½ mos. 10 yrs.	Excision
Calissano ⁸ Diss and Peterschmidt ¹⁹	1926 1926	63. 67	M M	Inguinal region Rt. lower eyelid	Hazelnut; reddish 1 x 1.5 cm.; ulcerated, hard	2 yrs.	Excised; recurred once
Grynfeldt ⁴³	1929	26	F	Lt. inguinal region	Linseed	5 yrs.	
Kehrer and von Jaschke ⁵³	1929	40	F	Lt. labium majus	Pea; had an opening externally	6 yrs.	
Hufschmitt and Diss ⁴⁸	1929	61	F	Scalp	Large; ulcerated, firm, violaceous	37 yrs.	Excised; recurred in 6 mos. with ad- hesion to skull and periosteum; regional metastases
Moriconi ⁶⁴	1931	59	M	Axilla	Walnut	6 mos.	Radical axillary dissection; metas- tases in regional nodes; well 5 yrs. later
Orol Arias ⁶⁸ Eichenberg ²⁶	1933 1934	30	F F	Rt. heel Labium majus	Ulcerated 0.5 cm.	3 yrs.	Excision left labium and left inguinal lymph nodes with metastasis; well 2 years later
Gougerot, Albeaux-Fernet and Dreyfus ⁴²	1934	43	F	Rt. leg	Hazelnut, hard, 25 cm.	3 mos.	Excised; died; no autopsy
Pelagatti ⁷⁰ Loos ³⁷	1934 1936	62 68	M M	Lt. upper lip Heel	Hazelnut Hen's egg	4 mos.	Excision
Fessler ³²	1939	55	F	Abdominal skin	6 cm.; reddish	Over 1 mo. (indefinite)	Excised

TABLE II
Tumors of Sweat Glands

Number	Age	Sex	Location	Size	Duration	Rate of growth	Remarks
<i>Syringoma</i>							
36-279 T. C. 272	26	M	Scalp	2 cm.	3 mos.		Syringoma but with atypical clinical and histologic features
<i>Hydradenoma Papilliferum</i>							
S-28-154	60	F	Back		Congenital		Hydradenoma papilliferum, discrete, partly solid growth, keratinized masses suggesting keratinization of ducts, upper corium
28-1805	38	M	Scalp		18 mos.		Hydradenoma papilliferum, discrete, solid as well as papillary growth, lower corium
29-2934	49	M	Head		Many yrs.		Hydradenoma papilliferum, discrete, simple structure, lower corium
31-1968	40	F	Abdomen				Hydradenoma papilliferum, discrete, cystic and solid, much degeneration of tumor, lower corium
35-974	65	F	Scalp	2 x 1.5 x 0.4 cm.			Hydradenoma papilliferum, complex papillary structure, both glands and ducts appearing to be involved, upper corium
38-996	45	F	Lt. infrainguinal region	Egg	3 yrs.		Hydradenoma papilliferum, discrete, lower corium
42-1293	54	F	Finger	3 cm.	6 mos.		Hydradenoma papilliferum, cyst with low papillary growth at periphery, in part suggesting apocrine gland, lower corium
42-2430	53	M	Neck	2 cm.	Congenital	Slow, past 10 yrs.	Hydradenoma papilliferum, some solid growth, some suggestion of apocrine gland, subcutaneous tissue
46296				2 cm.			Hydradenoma papilliferum, cyst without lining but with typical papillary structure lying free in center, subcutaneous
1280		F	Labium				Hydradenoma papilliferum, discrete, complex structure, lower corium
15707	40	F	Face				Hydradenoma papilliferum, encapsulated, intracanalicular growth, partly papillary but chiefly solid, some mucinous change in stroma, lower corium
<i>Hydradenoma Papilliferum Superficial</i>							
41-3362 10316	31	F	Forehead	2 x 2 x 3 cm.			Hydradenoma papilliferum, superficial Hydradenoma papilliferum, superficial

Hydradenoma Papilliferum Intermediata

		M	Lower lip	Several yrs.	Hydradenoma papilliferum, intermediate
		F	Near nose		Hydradenoma papilliferum, intermediate
			left cheek		
18-179	58				
42-413	35				
T. C. 525					

Hydradenoma

23-1570	52	F	Forehead	2 cm.	8 yrs.
25-1666	74	F	Scalp	Hen's egg	6½ yrs.
33-1879	40	M	Rt. supra-orbital	3 x 1.5 x 0.8 cm.	3 mos.
38-3223	58	M	Knee	3 x 5 cm.	10 yrs.
39-1518	20	M	Leg	2 x 2 cm.	2 yrs.
39-3141	46	M	Shoulder	Small pea	2 yrs.
40-1278	63	F	Rt. side of face	3 cm.	10 yrs.
41-4430			Scalp	2 x 1.5	
17-2		M	Forehead	x 1 cm.	
25-1982	40	F	Forearm	Large walnut	Since childhood
27-1113	33	F	Forehead		2 ± mos.
27-1419	23	M	Rt. thigh		
S-28-505	33	F	Finger	2 x 0.5	4 + yrs.
28-1910	55	F	Chest above breast	Pea	6 mos.
29-3067	31	F	Leg	Pea	10 mos.
30-397	24	M	Ear		Rapid
30-1416	57	F	Lip		
32-188	50-	M	Back of head		
T. C. 173	60				2 mos.
32-2401	60	M	Forehead	25 cent piece	
33-S-468	53	F	Over rt. eye	5 cm.	5 yrs.
33-S-822	34	M	Lt. hip	5 cm.	5 yrs.
			Rt. temporal region	3 x 2 cm. raised	
35-697	66	F	Scalp	5 cm.	15 ± yrs.
35-908	36	F	Rt. cheek	1 x 2 cm.	Activity past 6 mos.
35-1377	43	F	Forehead above eyebrow	Pea	2 yrs.
					16 mos.

Secretory (?) type
Secretory (?) type
Secretory (?) type
Secretory (?) type
Secretory (?) type
Secretory (?) type
Secretory (?) type
Duct (?) type, hyalin in tumor cells
Duct (?) type, hyalin
Duct (?) type, suggests an originally papillary growth
Duct (?) type
Duct (?) type, pedunculated
Duct (?) type
Duct (?) type, very cellular, few glands, much degeneration in stroma
Duct (?) type
Duct (?) type
Duct (?) type
Duct (?) type, hyalin
Duct (?) type, died 4 yrs. later, carcinoma of ovary with metastases
Duct (?) type, last seen 9 yrs. later, also separate basal cell carcinoma
Duct (?) type
Duct (?) type
Duct (?) type

TABLE II—(Continued)
Tumors of Sweat Glands

Number	Age	Sex	Location	Size	Duration	Rate of growth	Remarks
<i>Hydradenoma (continued)</i>							
36-S-565	67	F	Front of rt. ear	1.5 cm.	14 yrs.	Slow	Duct(?) type
36-996	83	F	Rt. ear	1 cm.	5-6 yrs.		Duct(?) type, entire corium
36-2541	68	F	Hand	Bean	10 yrs.		Duct(?) type
37-S-349	91	M	Rt. cheek	2 cm.	Pimple	Increase size past 6 mos.	Duct(?) type
37-1781	67	F	Temporal region		sev. yrs.		Duct(?) type
37-2332	45	F	Thigh	Pea	5-6 yrs.		Duct(?) type, some hyalin
39-1394	78	F	Below xiphoid		20 yrs.		Duct(?) type
39-1413	48	M	Upper arm		15 yrs.		Duct(?) type
40-438		F			Few yrs.		Duct(?) type
40-1003	51	F	Forearm	6 x 4 mm.	11 yrs.		Duct(?) type
T. C. 440							
41-485	35	F	Rt. temple	2 x 1.5 cm.	3 mos.		Duct(?) type
41-3856	45	F	Scalp	2 cm.	10 yrs.		Duct(?) type, entire corium, much hyalin; in part suggests keratinization where ducts approach epidermis
42-829	43	F	Ext. auditory canal at orifice	1" diam.	2 yrs.		Duct(?) type, entire corium, early hyalin
42-981	25	M	Below ear	$\frac{3}{8} \times \frac{1}{4} \times \frac{1}{8}$ "	20 yrs.		Duct(?) type
42-1529	72	F	Rt. lower eyelid	2 \pm cm. diam.	20 yrs.		Duct(?) type, whole corium
42-1555	65	F	Forehead		20 yrs.		Duct(?) type
78	45	F	Rt. thigh				Duct(?) type
13680	50	F	Arm	2 cm.	40 yrs.		Duct(?) type
35590	47	F	Neck				Duct(?) type, early hyalin
44499	70	F	Face				Duct(?) type, midcorium
45276a	64	M	Scalp	16.5 cm.			Duct(?) type
45276b	64	M	Back	5 cm.			Duct(?) type
46911	51	F	Arm	2 cm.			Duct(?) type
<i>Hydradenoma with Associated Hair Follicle Tumor</i>							
30-1513	73	F	Scalp				
30-2489	59	F	Lip	1.5 cm.	8-10 yrs.		Many cysts, fibrosis with calcification, subcutaneous
31-1789	35	F	Thigh	10 cent piece	4(?) yrs.		Fibrosis of stroma with much fat
32-1275	56	M	Nose	1 cm.	2 yrs.		Much fibrosis and fat in stroma
33-2494	31	M	Upper lip	$2\frac{1}{2} \times 2\frac{1}{2}$ "	16 yrs.		Chiefly duct type with some foci of tumor of hair follicle
34-1415	60	F	Sacroiliac region				

Hydradenoma with Associated Hair Follicle Tumor (continued)

35-1181	50	F	Rt. breast	Less than 25 cent piece	2+ yrs.	Growth apparently originating from sebaceous glands, hair and coil, some foci of keratinized cells; negative Hydradenoma papilliferum with basal cell carcinoma and hair matrix carcinoma, congenital Much fat in the stroma Some cysts, marked mucinous change in stroma
37-2022	43	F	Rt. temporal	50 cent piece	20 yrs.	
39-S-529	77	F	Abdomen	3 cm.	15 yrs.	
42-1205	38	M	Upper lip	$\frac{1}{2}$ cm.	Few yrs.	
15005	55	M	Scalp vertex	2.0 cm.		

Hydradenoma Arising from Apocrine Gland

26-1276	30	F	Labium	3-4 mm. 0.5 cm. 3-5 mm. diam. 0.8 cm.	1 yr.	
29-1556		F	Vulva			
37-943		F	Urethra			
39-1461	55	F	Below ear lobe			
40-3609	45	F	Labium			
13343	55	F	Labium rt.	3-5 mm. diam. 0.8 cm.		
30211	61	F	Labium, rt.			
37056	60	F	Near anus			

Mixed Tumors

C. S. 15-116	67	M	Nose	Pea	1 yr.	
36-2339	60	F	Temporal region	2 x 1 x 1 cm.	About 1 yr.	
37-838	62	M	Upper eyelid	1.5 cm.	Long	
37-2222	62	M	Scalp	1.5 cm.		
W635	38	M	Rt. hand	3.5 cm.		

Hydradenoid Carcinoma

32-627	73	F	Ear	1.5 x 1 x 0.5 cm. Hen's egg 6 cm. Marble 11 cm.	2 mos. Congenital 30 yrs. 2 mos.	Rapid past 6 weeks	Adenocarcinoma invading cartilage Adenocarcinoma with metaplasia suggesting basal cell carcinoma with foci of keratinization Invasive growth from hydradenoma papilliferum, ana- plastic part solid, much necrosis Hydradenoid carcinoma, parts of which resemble basal cell carcinoma, suggestion of origin from surface Hydradenoid carcinoma arising from hydradenoma papilliferum Hydradenoid carcinoma arising from hydradenoma papilliferum of apocrine glands with foci of hair matrix carcinoma; carcinoma <i>in situ</i>
T. C. 183	81	M	Face				
37-1814			Angle of scapula				
37-2023	76	F	Thigh				
39-809	80	F	Thigh				
39-1475	65	F	Thigh	11 cm.			
40-2059	62	F	Anus				
T. C. 454							

growth. A single recurrence was observed in four cases and there was no instance of metastasis. All of these tumors were excised.

One of our tumors (40-2059) deserves special mention. It was a cauliflower plaque completely surrounding the anus and measuring 11 cm. in greatest diameter. The growth did not extend into the canal. The epidermis covering the papillary growth was greatly thickened by active proliferation of cells which in part was atypical and represented carcinoma *in situ*. There was also invasive growth from the epidermis. Filling the corium was a tumor of sweat glands and of hair follicles, in part anaplastic. The tall acidophilic domed cells of the glandular portion suggested origin from apocrine gland. The malignant change in the epidermis and in the cutaneous appendages appeared to be independent.

SUMMARY

Tumors of the sweat glands are probably fairly common but they are rarely seen in the laboratory, as they are seldom removed except in those cases where they are unusually large, as they tend to be on the scalp; or where they are annoying because of their location, as on the vulva.

They tend to occur on certain parts of the body, such as the scalp, anterior chest, axillary folds, gluteal and perineal regions, and labia.

A congenital origin has been assumed by most writers in spite of the fact that with the exception of syringoma, which typically occurs at puberty, the majority of these tumors are noted first in adult life. The chief support of this view is the organoid histologic structure and the fact that some of them arise from several skin components.

There are two main histologic types, papillary and solid. The latter is more common. The solid forms may resemble basal cell carcinoma, but the pattern of growth is quite different and the alveoli lined by cuboidal epithelium embedded in solid cell masses make the origin clear. The origin of basal cell carcinomas from the sweat glands is unlikely on theoretic grounds and has yet to be proved. Differentiation of tumors arising from ductal portions of sweat glands as contrasted to alveolar is uncertain.

Carcinomas of sweat glands have been described on the basis of histologic invasive and anaplastic growth, but metastasis is exceptional. While there are rigid diagnostic criteria for hydradenoma and hydradenoid carcinoma, the failure to apply these uniformly has led to much confusion in the differential diagnosis.

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C. TUMORS OF SPECIALIZED SWEAT GLANDS

Tumors of specialized sweat glands, ciliary, apocrine, and ceruminous, are of theoretical interest, but so far as we know their histologic differentiation has no clinical importance.

1. Ciliary Glands

The ciliary gland or Moll's gland is a specialized sweat gland on the lids in close relation to the eyelashes. It differs in having a simpler coil structure and wider lumen and in opening near or into the hair follicle. According to Huber and Picena ⁴ an additional means of differentiation is an apocrine-like appearance of some of the cells of Moll's glands. Sweat glands of the ordinary sort are also found on the eyelid. We have no first-hand knowledge of tumors arising from Moll's gland but a few tumors have been reported. Two useful reviews are by Huber and Picena in 1932 and by Hagedoorn ³ in 1936. The earliest reported cases were those of Salzmann ⁷ and Wintersteiner.⁸

Few authors are willing to assert definitely that the tumors are of Moll's glands rather than of the ordinary sweat gland. Letulle and Duclos ⁵ wrote with a certain amount of confidence in their case, on the basis of absence of acinus formation, and the prominent myofibrils and elastic fibers around the tubular structure of the tumor. Coats ¹ felt that the absence of hairs in his tumor, an adenocarcinoma, would probably indicate that it was not from Moll's gland. Zeeman's ⁹ tumor, which appeared after the removal of a cyst from the eyelid, is not alto-

gether convincing; it resembles rather a basal cell carcinoma. Nichelatti⁶ and Huber and Picena⁴ described cystic adenomas. These tumors occur in adults and grow slowly. The rapidly growing tumor reported by Gault and Legait² seems to be exceptional in this respect. Recent rapid growth or, rarely, ulceration was the usual reason for operation.

All of the tumors have been considered as adenomas except that of Coats,¹ which he called adenocarcinoma on the basis of histologic invasive growth. Recurrences after surgical removal have not been reported.

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2. Tumors of Apocrine Glands

The apocrine glands occur principally in the axilla, the nipple, the genital and anal regions, and along the course of the embryonic milk line, but they may be found occasionally in almost any part of the skin. Some writers have classed them with the sex glands because of the fact that they develop at puberty and have been described as changing in size with the menstrual cycle. The secretory process is similar to that of milk. The general architecture resembles that of dilated sweat glands, but the epithelium is quite different. The cells are two to three times as large, and are tall cuboidal or columnar with a prominent, domed, free border. The cytoplasm is eosinophilic and frequently contains small fat droplets and pigment granules which react positively for iron.⁶ The myoepithelial cells are large. The lumen of the secreting portion is several times larger than that of the sweat gland.¹³

The general impression of the rarity of these tumors is probably due to the fact that they have been reported with tumors of the ordinary

sweat glands. Many of the hydradenomas of the vulva arise in apocrine glands. A few writers have attempted to demonstrate the origin of syringoma and epithelioma adenoides cysticum from apocrine glands.^{3, 7, 8, 12} Ewing⁵ does not discuss tumors of apocrine glands as such, but includes an illustration (Fig. 249) with the caption "Adenoma of axillary sweat glands. An occasional source of mammary carcinoma."

A clinically malignant carcinoma of the apocrine gland has not been reported so far as we know.* McDonald¹¹ recently reported twelve adenocarcinomas of the apocrine gland from the vulva. These differ from adenomas only in the mitotic activity and the microscopic invasive growth. It has been suggested that extramammary Paget's disease may in some instances be due to invasion of epidermis by tumor cells from an underlying carcinoma of apocrine gland but clear proof is lacking.^{2, 4, 9, 14} Creighton¹ associated "chimney-sweep's" cancer with the apocrine glands of the scrotum.

In our series of 121 tumors of cutaneous glands there are 9 from apocrine glands: 6 from the vulva, 2 from the anus and 1 from below the ear, a quite characteristic distribution. The tumors lie deep in the corium and are 1 to 2 cm. in diameter. The cells usually are typical throughout. Occasionally small foci of apocrine cells are seen in a tumor otherwise of the ordinary sweat gland type. The height of the cells varies considerably, but as a rule ranges from one and one-half to three times the width. The cytoplasm is homogeneous, moderately dense, sharply defined, and is intensely acidophilic. The cells at times contain a few fine vacuoles and a small amount of brownish to golden-yellow, finely divided, nonrefractile pigment, positive for iron, midway between the nucleus and the free edge. The nucleus, often round, may be ovoid and commonly lies about one-third of the distance from the basement membrane to the lumen. It is moderately rich in chromatin with a nucleolus of average mass.

The papillary cystadenomas have either a simple or complex structure. The vascularization is ordinarily poor, but there is virtually no evidence of hemorrhage or of degeneration. Other than in papillary projections, the tumor carries little or no stroma, merely expanding that portion of the corium in which it occurs. The contents of the cyst into which the papillary projections extend are usually clear without evidence of mucin, or degenerative products. Two of our tumors are solid adenomas and the others papillary, having the structure of hydradenoma. Two tumors, one solid and one papillary, have histologic malignant properties, active mitosis and invasive growth. Of particular

* The so-called sweat gland carcinoma of the breast¹⁰ will be described separately.

interest was a carcinoma *in situ* in the epidermis overlying the solid tumor. We are in doubt as to the propriety of considering any of the tumors of apocrine glands as carcinoma in view of their apparently invariable benignity.

Summary. Tumors of apocrine glands are not uncommon and occur chiefly on the vulva and near the anus. There is no clear proof that they are clinically malignant.

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3. Tumors of Ceruminous Glands

The ceruminous gland is individualized by a refractile pigmented secretion. In other respects it resembles the apocrine gland with all the characteristic features exaggerated: the glands are larger as are also the secreting cells with their domed free border; lipoid and pigment (iron-containing) are especially conspicuous and the myoepithelium is more prominent.

A very few tumors of these glands have been described. Brock² and Sprenger and Prietzel⁹ have reported adenomas of the secreting portion and Alonso,¹ in a general work on tumors of the ear, cited other cases. Carcinoma of the ceruminous gland would seem to be an extraordinarily

rare tumor. There are only two unequivocal cases in the literature: one case reported by Montpellier and Laffargue⁵ and that of Warren and Gates.¹⁰ Two others which may fall in this group are those of Sprague⁸ and of Yearsley and Butterfield.¹¹ Carcinomas of the ear canal, whose histology was not clearly specified, have been reported by Furstenberg,³ Richter,⁶ Schall⁷ and Lukens.⁴

We have one tumor of ceruminous gland, a carcinoma.¹⁰ It is of particular interest because of the gradations of proliferative change, from hyperplasia to highly malignant invasive carcinoma involving lymphatics, nerves and parotid gland. Even in the least differentiated portions there are some of the characteristics of the normal structure: excessive lipoid giving the cytoplasm a ballooned, reticulated appearance, and abundant pigment in powdery granules or in large aggregates, sometimes extruded from the cell.

The tumor presented as an ulcerated lesion in the groove behind the ear lobe rather than in the auditory canal. This superficial ulcerated portion of the tumor was the least differentiated; the less malignant portions and the glandular hyperplasia were near the normal ceruminous glands of the canal. The tumor had been noticed a year before it was removed and latterly had grown rapidly and resulted in facial paralysis. It measured 3 cm. in diameter and grossly was partly scirrhus and partly cystic. Although microscopically there was extensive lymphatic invasion, no gross metastases had made their appearance up to 6 months after removal.

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D. TUMORS ASCRIBED TO SWEAT GLANDS

1. *So-Called Sweat Gland Carcinoma of the Breast*

Since the term "sweat gland carcinoma" has been used to describe certain tumors of the breast, this group of carcinomas must be considered in a discussion of the tumors of sweat glands. "Sweat gland carcinoma" of the breast is said to be differentiated from carcinomas of mammary glands by the following features: location near the skin and hence a tendency to early ulceration and pain; a yellow color; and a histologic structure of columnar cells with acidophilic, opaque, somewhat granular, and sometimes fatty cytoplasm reminiscent of apocrine glands.⁷ For this last crucial feature to carry any weight one or more of the following assumptions is necessary: First, that sweat glands exist below the corium in mammary tissue. If this be true, the condition is very rare and is not found in the breast tissues seen in our laboratory. Second, that the resemblance of the cells of these mammary tumors—pale, acidophilic, and columnar—to those of apocrine glands implies origin from sweat glands. In pathologic conditions such as chronic cystic mastitis, such cells are frequently seen in ducts or acini continuous with readily recognizable mammary epithelium. Third, that breast glands are intimately related to sweat glands embryologically, and a reversion to the latter may occur. All of the skin appendages, as well as the mammary gland, derive from primitive surface epithelium independently. A more complete discussion may be found in Ewing.⁵ While the resemblance of the cells of some breast carcinomas to apocrine cells has long been recognized, the histogenetic relationship has not been established.

Cahen¹ first interpreted the cysts of the breast lined with eosinophilic, distinctly lipped cells as sweat gland retention cysts. Creighton² explained these eosinophilic cells as an anomalous reversion to the sweat gland type of epithelium. Delbet and Mendaro⁴ described this epithelium as "idrosadenoid." Herzenberg⁶ found in apparently pathologic breasts of a stillborn infant multiple cysts lined by eosinophilic epithelium which he believed were dilated apocrine glands. Von Saar⁹ thought such structures represented arrested differentiation of mammary tissue and Prym⁸ spoke of dedifferentiation or metaplasia to an embryologically earlier type of lacteal duct. Dawson³ suggested that they represent a "past proliferative phase."

Conclusion. We do not recognize the so-called sweat gland carcinoma of the breast as being a true tumor of sweat glands but rather as a tumor of mammary epithelium.

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2. Turban Tumors

The so-called "turban tumors" of the scalp are benign, multiple, largely confined to the scalp, and of slow growth. They typically appear at puberty or in early adulthood, especially on females, and are familial. Ancell² reported a case in which tumors appeared in 4 generations of a single family. The epithelial pattern is variable depending on the structure from which the tumor originates. The rare sarcomas of the scalp^{3, 12, 27, 33, 35, 48} should not be included in this group.

In the early literature^{23, 31, 45} these tumors were referred to as endo-theliomas, as were most of the nonkeratinizing epitheliomas of the skin before Krompecher's²⁸ definitive work. Ewing¹⁸ discussed the endo-thelial versus epithelial characteristics of the tumors. The term "nevoid" has been considered suitable inasmuch as it indicates the embryonic or congenital nature of the tumors which both the clinical manifestations and organoid histologic structure suggest.^{8, 14, 24, 29, 40, 46, 51, 53} Macleod³⁰ and Binkley⁸ considered that these tumors arise from embryonal rests and are relatively undifferentiated ectodermal structures which may resemble hair follicles or glands. Ewing pointed out the histologic resemblance of turban tumors to cylindromatous tumors of salivary and lacrimal glands and mucous glands of the nasal septum, accessory sinuses, and other locations. All of these tumors are composed of small hyperchromatic cells which tend to have an organoid

arrangement. But the point of particular interest which they have in common is the characteristic hyaline and sometimes myxomatous alteration, as well as the fibroblastic proliferation of the surrounding stroma. Opinion has been fairly evenly divided among writers as to whether the tumors are sebaceous,^{5, 10, 38, 39} derived from coil glands,^{4, 11, 26, 37, 47} or from hair follicles.^{6, 8, 34, 42, 44, 49, 50, 51} The fact that they are generally considered to be of the basal cell type seems most important.^{1, 6, 8, 9, 14, 17, 18, 19, 21, 25, 28, 29, 30, 32, 36, 40, 41, 44, 46, 49, 52, 53}

Some of the terms which have been applied to multiple tumors of the scalp in the past are as follows: benign cystic epithelioma of the scalp;¹⁵ benign multiple epithelioma of the scalp;¹⁶ familial cylindroma;⁴³ sebaceous tumors of the scalp;³⁸ primitive sebaceous epitheliomas;⁵ cylindroma;⁷ trichadenoma cylindromatosum;¹¹ naevus epitheliomato-cylindromatosus;⁸ carcinoma basocellulare hyalinicum;²⁸ hyaline-like sweat gland epithelioma;³⁷ multiple sudoriparous adenoma;⁴ syringoma of the scalp;¹⁹ sweat gland carcinoma;²⁶ benign multiple basal cell epithelioma;¹ congenital endotheliomata;²⁷ multiple endotheliomas of the scalp;³¹ endothelioma of the skin and turban tumors;⁴⁵ multiple sarcoma;¹² withering sarcoma;³ confluent tumors of the scalp;³⁵ naeoeptithelioma adenoides.²⁴ In spite of this diversity it seems clear that most authors agree on certain main points:

1. The tumors are multiple, benign, or, at most, of histologic malignancy only.

2. The histologic features are variable within the limits of basal cells or the cells of cutaneous appendages.

3. The cells are nonkeratinized, with few exceptions.

4. Cysts, hyalinization of stroma, and frequently cylindromatous arrangement occur.

5. Ulceration or fixation is rare.

The frequency of these tumors in relation to other epithelial tumors of the skin cannot be determined with certainty, partly because of the lack of uniformity in the interpretations of different observers. Ronchese³⁹ accepted 31 cases from a total of 50 reported in the literature. This seems far too low an incidence.

Patients do not come for treatment until the tumors cause inconvenience, which, because of their very slow growth, is a matter of many years. In the reported cases, the average duration of the tumors before treatment was 25 years and the average age of the patients at this time was 53 years.

The first lesions appear most frequently at the hairline of the temple and forehead as smooth, firm, nontender nodules with no discoloration of the normal, overlying skin. They are freely movable beneath the

skin and in the deeper structures. As time goes on, other nodules appear. These apparently develop as multiple, discrete foci rather than by infiltration from the primary nodule. The tumors often cover the entire scalp and may extend later around the eyes, ears, neck and upper part of the chest. Those on the nonhirsute parts of the skin are usually smaller and fewer in number.

One of our tumors of coil gland (45276) was entirely typical of the so-called turban tumor. The tumor had first been noticed on the front of the scalp in early manhood and had continued to grow slowly. Occasional trauma had caused bleeding and infection from time to time. After 40 years, the tumor presented as a lobulated mass covering the anterior half of the scalp and encroaching on the forehead and laterally behind the ears. The lobules were 3 to 5 cm. in height. Another elevated lesion, 2 cm. in diameter, was present on the back. Both had similar microscopic structure. Similar lesions were described in 4 generations of the family—the great-grandfather, mother, sister, and son. But none of 5 grandchildren was affected.

Excision is the usual method of treatment,^{21, 22, 36} but because the tumors are so easily enucleated, the margin of excision is often not wide enough. There may be recurrence. Schuermann and Weber⁴⁴ advised supplementary radium therapy in some cases. Binkley⁸ used interstitial radium in one case. Binkley,⁸ Eller,¹⁷ Delaye, Hugonot and Lacasagne,¹⁴ and others have reported x-rays as ineffective. Because of the fact that tumors are usually numerous, Delaye, Hugonot and Lacasagne advocated galvanocautery. There has been no reported instance of malignancy developing in a turban tumor. Binkley⁸ and de Beurmann¹³ have reported spontaneous regression.

Summary. The turban tumor is not a special histologic entity and may resemble various slowly growing epithelial tumors of the skin and its appendages. It has been generally recognized that the clinical and histologic characteristics are those of a benign organoid tumor.

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3. *Mixed Tumors of the Skin*

The few reports of cutaneous mixed tumors in the literature have been reviewed recently by Scharla⁴ and Simard.⁵ The tumors have been reported from the hand, finger, lower leg, thigh, scalp, and flexor surface of the elbow. The diagnosis is not always simple. The typical basal cell carcinoma and some of the tumors of skin appendages frequently have very marked hyaline and myxomatous changes which may simulate cartilage and osteoid. The problem is well illustrated in the report by Geschickter and Koehler² who described certain cutaneous mixed tumors believed to originate in aberrant salivary gland. From the description and photographs it would be difficult to differentiate them from basal cell carcinomas with cystic and hyaline stromal changes. Simard considered his case a mixed tumor of sweat gland.

We have had five tumors of the skin in which cartilage or osteoid tissue and bone, or all three, made up an important part of the tumor. One grew on the hand and four came from the head. In all of the tumors the neoplasm developed in part from the coil glands; in three, hair follicles were involved as well and in one there was proliferation of sebaceous glands. Fibroblastic proliferation of the stroma with mucinous and hyaline change was prominent in all of the tumors.

As in the case of the tumors of salivary gland, it is an open question whether cutaneous mixed tumors are true teratomas or slowly growing epithelial tumors in which metaplasia of epithelium and/or stroma occurs.^{1, 3}

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DESCRIPTION OF PLATES

PLATE 70

- FIG. 1. Hyperplasia of ducts of sweat glands. Congenital lesion. Hematoxylin and eosin stain. $\times 15$.
- FIG. 2. Hyperplasia of secretory alveoli of sweat gland accompanying an inflammatory lesion. Hematoxylin and eosin stain. $\times 225$.
- FIG. 3. Metaplasia of secretory alveoli. Hematoxylin and eosin stain. $\times 375$.

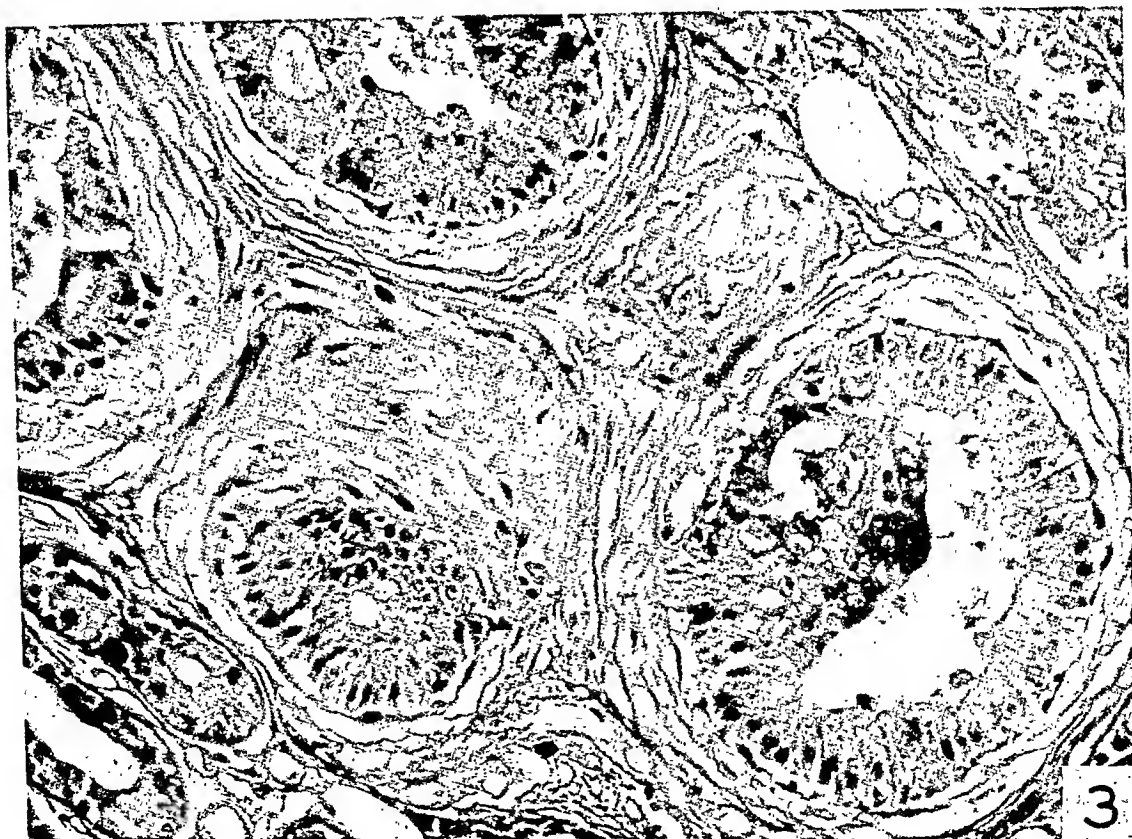
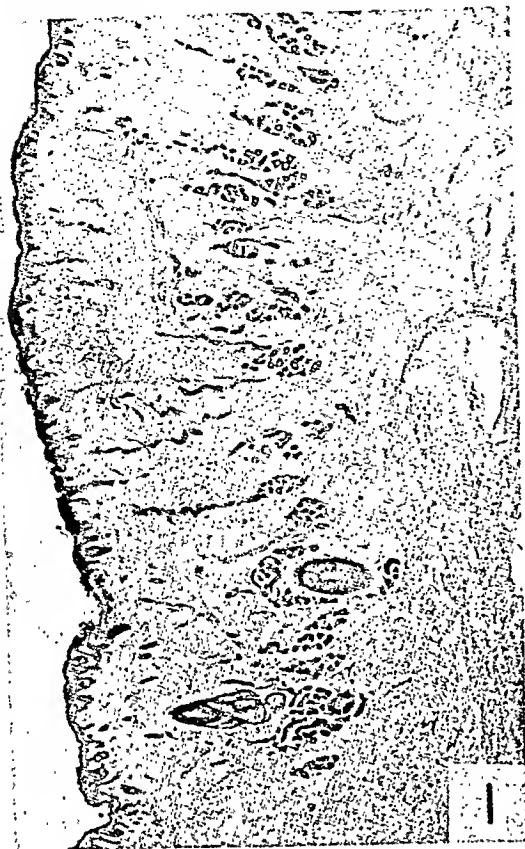


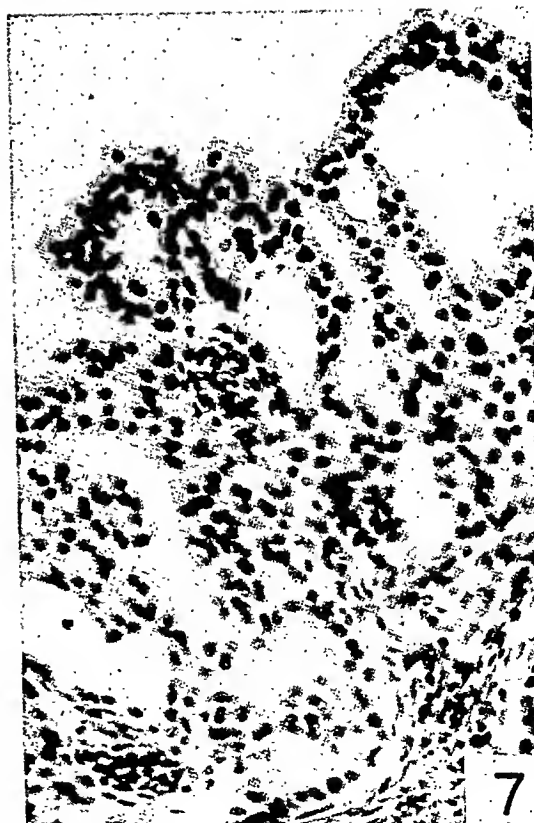
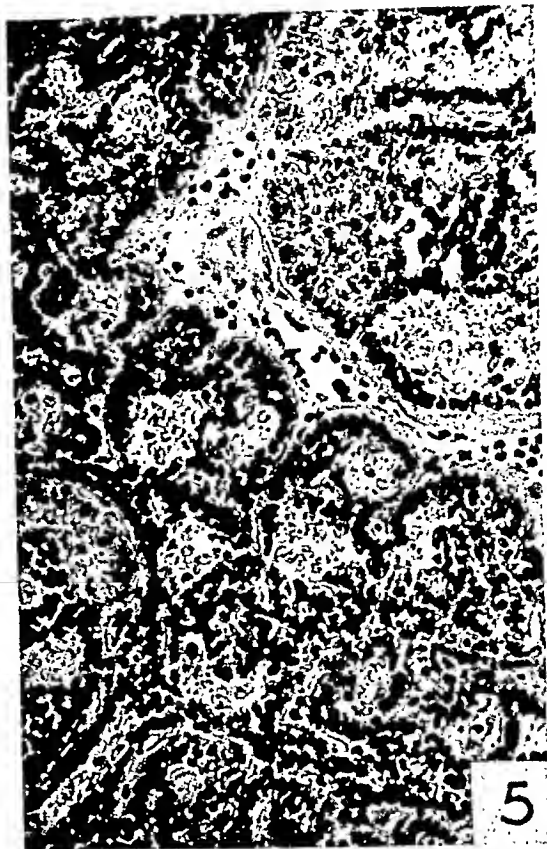
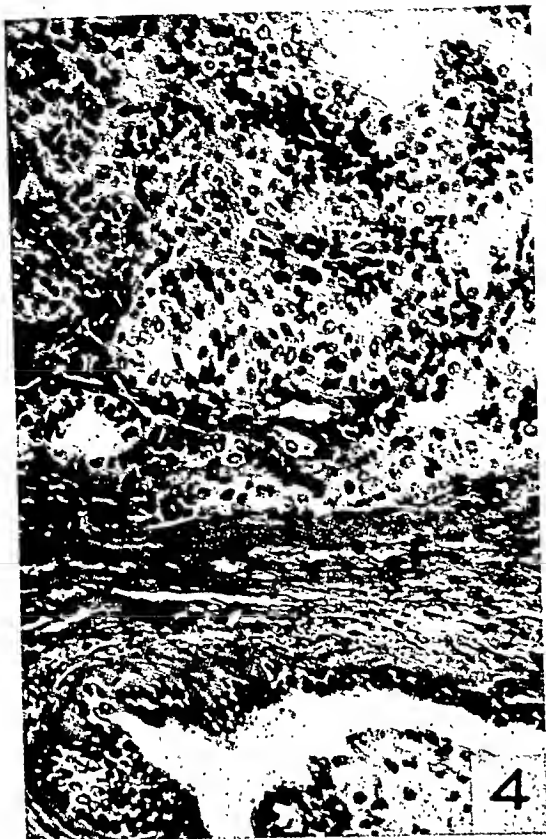
PLATE 71

FIG. 4. Hydradenoma papilliferum. Clear reticulated cells resembling sebaceous cells are seen in the lower right-hand corner. Tumor had been present on the neck since childhood and measured 2 cm. in diameter. Phosphotungstic acid hematoxylin stain. $\times 265$.

FIG. 5. Hydradenoma. There is an alveolar pattern. Pale cells are surrounded by darker smaller cells and there is an early hyaline deposit in the basement membrane. Phosphotungstic acid hematoxylin stain. $\times 265$.

FIG. 6. Hydradenoid carcinoma. Hematoxylin and eosin stain. $\times 250$.

FIG. 7. Hydradenoma papilliferum of apocrine gland. Hematoxylin and eosin stain. $\times 210$.



Gates, Warren and Warvi

Tumors of Sweat Glands

PLATE 72

FIG. 8. Hydradenoma papilliferum. The cavity of the lesion opens onto the surface at the upper right, and there is slight metaplasia of ducts and epithelium. Superficial type. Hematoxylin and eosin stain. $\times 165$.

FIG. 9. Hydradenoma papilliferum. Intermediate type. Hematoxylin and eosin stain. $\times 165$.



GYNANDROBLASTOMA OF THE OVARY*

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The term gynandroblastoma was first used by Robert Meyer¹ in 1930, in the discussion of a series of arrhenoblastomas reported by him, one of which had in part a histological similarity to granulosa cell tumor and was accompanied by uterine hypertrophy. He suggested that ovarian tumors may arise from indifferent elements which become morphologically and functionally hermaphroditic so that they may cause both hypertrophy of the uterus and masculinization.

Plate,² in 1938, collected 12 cases from the literature, which he believed could be placed in this category, and added one of his own. The tumor in his case had both granulosa cell and arrhenoblastoma elements, partly separated, but with visible transitions between them. He distinguished this tumor on histogenetic and morphological grounds from granulosa cell tumors and arrhenoblastomas.

No collected data concerning the biological effects of such tumors exist, but as certain clinical and pathological differences from the arrhenoblastomas might be expected to occur, we have reviewed the cases cited by Plate² together with other pertinent cases in an effort to determine whether or not such a distinction is supported by biological evidence, in connection with the additional case herein reported.

Although Plate's² reference is to Meyer's¹ case 6, it is his case 7 to which Meyer referred in his use of the term gynandroblastoma. Meyer stated, however, that his cases 6 and 7 are very similar and belong to the same group, with identical masculinizing features.

The following case was originally considered as an arrhenoblastoma, but the diagnosis was later changed to gynandroblastoma on the basis of both clinical and histological evidence.

REPORT OF CASE

The patient, a white woman, 24 years of age, came to the hospital seeking relief from lower abdominal pain and continuous vaginal bleeding of 10 days' duration. Menstruation began at 14 years and had always been regular, with 28-day intervals until the last 4 months before hospital admission, when she had flowed for 5 to 7 days about every 2 weeks. She was married at 18 years of age, with one pregnancy ending in abortion. She was divorced at 20 years and had remarried 5 months before hospital admission. Libido had never been strong. Physical examination revealed hirsutism with male voice and beard, acne vulgaris and hypertrophy of the

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clitoris, these changes having developed in the past 5 years. The breasts were slightly pendulous but not atrophic (Figs. 1 and 2). Her blood pressure was 118, systolic; 85, diastolic. A large pelvic tumor was palpable on the left side. A single 24-hour urine specimen collected before operation was tested for androgenic hormone by Dr. R. G. Gustavson, using the rat seminal vesicle method, with negative results. He also extracted approximately one-half of the tumor and tested the extract for androgenic effect, in the same way, with negative results.

At operation (by E. A. M.) the tumor was found to be associated with the left ovary. It was encapsulated but adherent to the posterior pelvic wall. Removal of the tumor together with the left adnexa was effected. The uterus was large and soft. The right ovary was small. Recovery was uneventful.

Following the operation vaginal bleeding ceased. Curettage was not performed. A normal menstrual period occurred 1 month after operation and menstruation has been normal since that time, for 3 years. The patient has been well except for one attack of pyelitis.

At the present time her voice is less hoarse than formerly but is still deep in pitch. The clitoris remains hypertrophied. Hair growth on the face and legs is less rapid and not so conspicuous. She has gained about 15 pounds and notes that her breasts are now less pendulous (Fig. 3).

Pathological Description

The tumor was spheroidal, 11 cm. in diameter, and weighed 349 gm. It was enclosed in a smooth fibrous capsule which was intact except for a roughened area 2 cm. in diameter where the tumor was adherent to the pelvic peritoneum.

The fallopian tube and ovary were attached to the tumor. The tubal attachment consisted of the mesosalpinx, while the ovary was incorporated in the capsule of the tumor, indicating that the tumor was situated in the mesovarium. The ovary was compressed into the form of a cap measuring 4.5 cm. in diameter, by 2 cm. in thickness at the center, tapering toward the edges, where the tunica albuginea became continuous with the capsule of the tumor.

Surfaces made by cutting through the ovary and tumor showed the ovarian cortex to be thin, grayish white, and solid except for several small cysts filled with clear fluid. Numerous cysts, ranging from less than 0.1 cm. to 0.4 cm. in diameter, and containing thick, turbid gray secretion, were present immediately beneath the capsule, at its junction with the tunica albuginea. These became less numerous and disappeared from the capsule entirely about 5 cm. beyond the junction ring. The substance of the tumor was solid, soft and moderately friable. It was coarsely mottled with pale yellow and white areas, and the central portion, comprising about one-third of the tumor, was dark purplish red as the result of hemorrhagic infiltration (Fig. 4).

Microscopical examination of paraffin sections of the ovary stained with hematoxylin and eosin disclosed numerous primordial follicles and a few developing follicles, but no corpora lutea. Corpora albicantia and

several small atretic follicular cysts were seen. The cortical stroma was scanty. The medulla was spread out, extending laterally to merge with the inner layer of fibrous tissue forming the capsule of the tumor. This layer was composed of loosely knit, thin collagenous fibers with interspersed fibrocytes. It carried numerous blood and lymphatic vessels and several nerve trunks.

Beginning within the medulla of the ovary and extending outward from the hilus along the inner layer of the capsule of the tumor there was a series of epithelium-lined tubules, corresponding to the cysts described grossly. They were of variable caliber and outline, some being distended with either granular or hyaline eosinophilic substance (Fig. 5). The epithelium was cuboidal in some and columnar in others. It was nonciliated but had a definite cuticular border. The cytoplasm was basophilic, with small vacuoles or with a single large vacuole located above the nucleus. The nuclei were large, oval and coarsely reticular. These tubules were located between the capsule and the substance of the tumor and did not extend into the deeper portion of the tumor.

The tumor was composed chiefly of more or less fusiform cells with large reticular nuclei containing much chromatin, and scanty basophilic cytoplasm, supported by a thin meshwork of fibrillary connective tissue, partially hyalinized in some areas, with many small blood vessels.

There was a distinct lobular arrangement throughout the entire tumor, the lobules being partly separated by thin fibrous septa. Viewed grossly or in low-power magnification the lobules were seen to differ in appearance, some being homogeneous and well circumscribed, while others were ragged in outline and uneven in density (Fig. 6). Under higher magnification the homogeneous lobules were found to consist of fusiform cells in poorly defined fascicular grouping with numerous zones in which the cells became polyhedral. The arrangement here resembled that of the relatively undifferentiated type of granulosa cell tumor (Fig. 7).

The other lobules were composed of similar cells but with distinctly different arrangement. In these, the cells formed tight whorls, solid cords with the cells curved across the long axis, or plicated tubules with incomplete lumina (Fig. 7). Transition zones between two types of lobules were quite frequent.

Groups of larger cells with abundant brownish red cytoplasm appeared in and about the second type of lobule. They resembled the interstitial cells of Leydig of the testis. In frozen sections stained with scharlach R they were found to contain lipoids in large quantity. Similar groups of lipoid-loaded cells were found in the connective tissue near the subcapsular epithelium-lined tubules (Fig. 5). In the homogeneous

lobules there were occasional individual cells containing lipoids, but they were smaller, with less cytoplasm, and the lipoid stained less intensely than in the interstitial cell groups. The necrotic portions of the tumor contained many small lipid globules.

Sections stained by Masson's trichrome method and reticulum stains (Foot modification) did not serve to distinguish further cellular features.

Summary of Case

The clinical features of this case suggest a combination of the hormonal effects found in granulosa cell tumors and in arrhenoblastomas. It is to be noted that the patient continued to have recurrent uterine bleeding, without atrophy of the breasts and without loss of feminine body contour, during the time that hirsutism, voice changes and hypertrophy of the clitoris were developing. This is taken to indicate that both estrogenic and androgenic hormones were being elaborated by the tumor, with concurrent biological effects.

The histological structure of the tumor, with its distinctive lobular forms resembling granulosa cell tumor and arrhenoblastoma respectively, supports the biological evidence.

Bio-assay of the tumor substance and of the patient's urine failed to reveal the presence of androgenic hormone. However, since androgenic effects were well defined in the patient, it may be assumed that insufficient quantities of test material were supplied. The prompt re-establishment of normal menstrual function proves that the tumor was responsible for its disturbance. Hirsutism has diminished but has not disappeared. The voice remains masculine, and the hypertrophy of the clitoris persists.

The peripherally located tubules lined by columnar epithelium found in and near the hilus of the ovary are distinct from the rete ovarii. They are not characteristic of either granulosa cell tumors or arrhenoblastomas but have been noted before in arrhenoblastoma by Kanter and Klawans³ together with embryonic rete, tissue resembling spermatid ducts, mesenchyme and cartilage.

The presence of interstitial (Leydig) cell groups also has been noted in arrhenoblastomas (Kanter and Klawans³ and Novak⁴) although, according to Novak, their presence or absence does not always parallel that of virilism. These cells contain abundant lipoids and do not resemble degenerating cells undergoing fatty metamorphosis. It seems probable that they are concerned with elaboration or storage of the androgenic hormone. Stewart, Bell and Roehlke⁵ have shown the relationship of interstitial cell tumors of the testicle to the development of secondary sexual characteristics of the male.

REVIEW OF THE LITERATURE

The original publications referred to by Plate ² were reviewed. Additional cases, such as those reported by Bergstrand,⁶ were also studied in order to determine if possible whether any constant clinical features were common to these cases, and whether special histological characteristics could be found. No attempt was made to include all the reported cases of granulosa cell tumor and of arrhenoblastoma.

In several of the cases clinical data were lacking, and in others histological description was incomplete. The available data are included in the following summary.

Meyer, R.,¹ Case 6

Age. 25 years.

Menstrual History. Menstruation regular, 3 days with pain. Thyroid operation at 25 years, followed by amenorrhea for 2 years. Hemorrhage of 8 days' duration recurred 1 month later, with rapid increase of size of abdomen.

Pregnancies. Not stated.

Masculinization. Marked increase in hair growth, male distribution, all over body. Beard grew fast. Voice rough and lower. Clitoris hypertrophied. Body slender and emaciated. Vagina narrow and firm.

Postoperative Condition. Decrease in hair growth. Menstruation returned after 4 weeks and remained regular. Voice more feminine. Libido returned to normal.

Ovarian Tumor. Right, kidney-shaped, smooth, adherent, weight 4,450 gm. In one small, firm part there was one large cyst and multiple small cysts. There were also a few solid accumulations, yellow or dark red. Slides showed loose fibrous areas with uncharacteristic strands and small cysts with low epithelium. In most places epithelial strands subsided into loose sarcoma-like proliferation. The epithelia were characterized by very irregular forms influenced by surroundings. Single cells, then many cells, became excavated, forming cysts. This peculiar cyst formation was considered important and partly due to softening within sarcoma-like cell proliferations. In another part, small and large cysts were embedded in a network of delicate moist connective tissue without solid cellular proliferation. There were many connections between cystic areas and solid areas containing cysts. Therefore, the question was raised whether the cysts arose within areas of solid proliferation or from strands of similar epithelium, or were they a peculiar part of the tumor with a different origin? The better preserved epithelial cells, especially the cylindrical, gave a mucin reaction. The solid epithelial strands contained small cavities and were, moreover, in connection with the spaces lined by mucinous epithelium. There was no transition from solid tumor into mucoid epithelial cysts and no evidence of transformation of areas with mucoid epithelium into solid strands. Meyer believed that originally two separate parts must have existed. The formation of mucous cysts could not be compared to pseudomucinous cystadenoma. Cell forms were different, papillae were lacking, mucus was not abundant.

Uterus and Adnexa. Uterus and left ovary, atrophic.

Meyer, R.,¹ Case 7

Age. 35 years.

Menstrual History. Began at 16 years. Regular for 6 years, then irregular. Four children. Menstruation ceased for 6 years after fourth childbirth. Irregular hemorrhage and almost continuous bleeding in past 9 months.

Masculinization. Voice low-pitched, hirsutism.

Postoperative Condition. Voice low, hirsutism gone, breasts normal, libido normal.

Ovarian Tumor. Right, size of fist, firm, partly hemorrhagic. Solid cell cords and large epithelial areas interspersed with larger vessels with much connective tissue. Resembled the granulosa cell type.

Uterus and Adnexa. Uterus removed with the tumor. Hypertrophy of corpus uteri. No histological examination.

Tietze,⁷ Case 10

Age. 57 years.

Menstrual History. Amenorrhea from 40 to 50 years of age. Then menstruated regularly for 7 years. Last menstruation $4\frac{1}{2}$ months before admission.

Pregnancies. Five, the last 25 years before.

Masculinization. None.

Ovarian Tumor. Left, cut surface showed central, firm, whitish tumor, hazel-nut sized. Fusiform and polyhedral cells in islands. Cell types were readily distinguishable, but there were transitions.

Uterus and Adnexa. Preoperative curettage showed cystic glandular hyperplasia of the endometrium. Total hysterectomy, uterus normal size, with high mucosa, hemorrhagic. Right ovary senile.

Tietze,⁷ Case 11

Age. 76 years.

Menstrual History. Menses regular, menopause in 1904. Slight hemorrhages in 1924, subsiding after removal of polyps of the cervix. Slight hemorrhages in April, 1928, increasing after 2 months.

Pregnancies. Four.

Masculinization. Not described.

Postoperative Condition. Not described.

Ovarian Tumor. Left, size of hen's egg, solid. Right, firm tumor size of goose egg (fibroma). Left ovarian tumor made up of closely packed polyhedral cells and small groups of lighter cells between them.

Uterus and Adnexa. Size not stated. Myomatous nodules present. Endometrial polyps. Endometrium hyperplastic, with cysts.

Tietze,⁷ Case 12

Age. 74 years.

Menstrual History. Menopause 24 years before. Periodic slight hemorrhages lasting 3 to 4 days at irregular intervals for about 6 months.

Pregnancies. 11 births, 2 abortions.

Masculinization. Not described.

Postoperative Condition. Not stated.

Ovarian Tumor. Left, size of walnut, soft, white, with two parts, one densely packed with sarcoma-like cells with little connective tissue, the other with many gland-like lumina in large or small groups. There were transitions from one part to the other and a few follicle-like structures were present.

Uterus and Adnexa. Uterus size of fist, two nodes resembling myomas, two endometrial polyps.

Amati,⁸ Single Case

Age. 27 years.

Menstrual History. Amenorrhea complete for 5 years. Blood loss of the menstrual type then became re-established, becoming very abundant and accompanied by abdominal pain.

Pregnancies. None. Patient was virginal.

Masculinization. None.

Postoperative Condition. Dead.

Ovarian Tumor. Left, in part resembling testicular adenoma tubulare, in part lobular with islets and cysts resembling graafian follicles. Solid carcinoma with large cells and ovarian adenoma tubulare found united, for the first time, in this case. Not an ovotestis but a blastoma of germinal epithelium in a broad sense.

Uterus and Adnexa. Uterine mucosa presented characteristic pregravid modifications, with a wide decidual zone.

Schiller,⁹ Case 3

Age. 22 years.

Menstrual History. Began at 15 years, regular. Then irregular for 3 years, sometimes missing for several months, very scanty. Last period 7 months before.

Pregnancies. None.

Masculinization. Marked hairiness of chin, chest, and about umbilicus. Breasts very small. Face had masculine appearance.

Postoperative Condition. Recovery. Ten years later, genitalia normal, regular menstruation of 4 days' duration.

Ovarian Tumor. Left, cystic, size of child's head. Tumor half cystic, half soft hemorrhagic tissue with small islands, trabeculae and follicular zones with central cavities.

Uterus and Adnexa. Uterus not described, right ovary normal.

Schiller,⁹ Case 4

Age. 25 years.

Menstrual History. Regular until 2 years before. Irregular for 1 year. Amenorrhea for 1 year.

Pregnancies. Not stated.

Masculinization. Gain in weight (56 Kg.). Slight hypertrichosis, pubic hair slightly of male type.

Postoperative Condition. Recovery with loss of weight. Two months postoperatively, menstruation of 1 day's duration. Progynon given for 1 month, with menstruation for 4 days, followed by amenorrhea with increase in weight. Condition remained the same. No menstruation without medication.

Ovarian Tumor. Right, size of apple, yellow, firm, homogeneous. Partly composed of solid cells in hyaline connective tissue. Epithelial cells with round nuclei formed Call-Exner corpuscles similar to granulosa cell masses in other cases.

Uterus and Adnexa. Uterus not described. The left ovary contained a cyst the size of a goose egg, resembling the pseudomucinous variety. Cyst, only, was removed.

Schiller,⁹ Case 11

Age. 37 years.

Menstrual History. Began at 14 years and became irregular after birth of second child 13 years ago. Menstruation became more and more rare. In past 2 years only two menstruations.

Pregnancies. Two.

Masculinization. Trace of brown mustache, almost male voice.

Postoperative Condition. Recovery, well after 9 years. Had uterine myoma.

Ovarian Tumor. Right, size of an apple. Ascites. Tumor was trabecular with oval cells with epithelioid nuclei. Transition between fibrous and cellular areas. Call-Exner arrangement of cells present. Similar to mature granulosa.

Uterus and Adnexa. Not described.

Eerland and Vos,¹⁰ Case 2

Age. 35 years.

Menstrual History. Menopause 2 years before.

Pregnancies. Two children.

Masculinization. Virile body build, beard, male type of pubic hair, large clitoris.

Postoperative Condition. Menstruation set in 5 weeks after operation.

Ovarian Tumor. Right, size of child's head, believed to be arrhenoblastoma on grounds of clinical features, but resembling granulosa cell tumor histologically. Histological picture failed to reveal distinct tubular structures. Not entirely like other granulosa cell tumors with masculinization; in fact it was not to be distinguished from granulosa cell tumors without masculinizing features.

Uterus and Adnexa. Not described.

Eerland and Vos,¹⁰ Case 5

Age. 40 years.

Menstrual History. Menopause for years.

Pregnancies. Yes, number not stated.

Masculinization. Beard.

Postoperative Condition. Soon after operation growth of hair on the face disappeared and menstruation set in.

Ovarian Tumor. Right, solid, size of child's head. Solid, trabecular and folliculoid parts. Granulosa cell tumor or andreioblastoma?

Uterus and Adnexa. Not described.

Eerland and Vos,¹⁰ Case 13

Age. 45 years.

Menstrual History. Not stated.

Pregnancies. Not known.

Masculinization. Not described.

Postoperative Condition. Not described.

Ovarian Tumor. Left, only pieces examined. Solid tumor, both diffuse large cellular masses as in granulosa cell tumor and alveoli composed of larger light cells in epithelioid arrangement. Andreioblastoma(?). Malignant(?).

Frankl,¹¹ Single Case

Age. Young.

Menstrual History. Not stated.

Pregnancies. Not stated.

Masculinization. Markedly virilized.

Postoperative Condition. Not described.

Ovarian Tumor. Cystic, at first appeared to be a polycystic granulosa cell tumor. Only on examination of many areas was a small number of cords found comparable to those in arrhenoblastomas, the bulk consisting of granulosa cell tumor.

Uterus and Adnexa. Not described.

Bergstrand,⁶ Case 1

Age. 22 years.

Menstrual History. Began at 13 years, regular until age 20, then ceased.

Pregnancies. None.

Masculinization. Hirsutism appeared at the time menses ceased.

Postoperative Condition. Not stated.

Ovarian Tumor. Two ovarian tumors shaped like ovaries, cystic. Made up of cells similar to granulosa cells in smaller or larger units with cysts. Some in the form of cell cords. Cystic units had definite follicular character, many converted

into corpora atretica. Ova were found in some cysts. No corpus luteum tissue was found, but in large cysts there were intensely yellow nodes in the walls resembling corpus luteum. The cells contained fat, not found in other cells.

Uterus and Adnexa. Not described.

Bergstrand,⁶ Case 2 (Berners's)

Age. Not stated.

Menstrual History. Not stated.

Pregnancies. Not stated.

Masculinization. Hirsutism.

Postoperative Condition. Not stated.

Ovarian Tumor. Tubular structures interspersed with lipoid-containing cells, yellow color. Epithelial cells forming small cysts. Corpora atretica not seen.

Uterus and Adnexa. Not described.

Bergstrand,⁶ Case 3 (Strassman's)

Age. Not stated.

Menstrual History. Not stated.

Pregnancies. Not stated.

Masculinization. Hirsutism.

Postoperative Condition. Not stated.

Ovarian Tumor. Made up partly of tubules, partly of solid narrow cords of epithelial cells, and, between these, large lipoid-containing interstitial cells were present. The epithelial cells had undergone fatty metamorphosis to a certain degree and were not readily distinguishable from interstitial cells. There were many mitoses. Large hyaline areas like corpora atretica were found, but identity could not be proved.

Uterus and Adnexa. Not described.

Bergstrand,⁶ Case 4

Age. 37 years.

Menstrual History. Menses ceased 2 years before hirsutism appeared.

Pregnancies. One child.

Masculinization. Hirsutism.

Ovarian Tumor. Right, size of hen's egg. Round, yellow focus, size of walnut, and several cysts filled with brownish red masses. Very complex microscopically. The walnut-sized focus was made up of mucus-producing cylindrical epithelium surrounded by cellular connective tissue, similar to normal uterine mucosa; gave the impression of malformation of Müller's duct. No smooth muscle was present. Elsewhere the tumor was quite different, with large accumulations of two types of cells, with cysts. One cell type was large, rich in cytoplasm with large, round nuclei. The other was a small dark cell poor in cytoplasm. The dark cells formed garlands, with necrosis and hyalin.

Uterus and Adnexa. Left ovary slightly enlarged, contained small cysts.

Bergstrand,⁶ Case 5

Age. 17 years.

Menstrual History. Had never menstruated.

Masculinization. Hirsutism, voice change.

Postoperative Condition. Died 5 months after operation, with peritoneal metastases.

Ovarian Tumor. Right, size of child's head, soft, white. Left ovary similar but smaller. Bulk of tumor was made up of epithelium-like cells growing in diffuse masses separated by fibrous cords, with strand-like arrangement in some areas. Nuclei were vesicular, rich in chromatin with large nucleoli, relatively small amount

of cytoplasm. There were many mitoses. Cells of another form appeared lighter, with smaller nuclei. These were grouped about vessels, frequently showing fatty metamorphosis. The cytoplasm was variable. These cells resembled lymphocytes, but were not lymphocytes. Some cells formed garlands and tubules, and produced mucin. Similar conditions as in case 4, but not as clear-cut.

Uterus and Adnexa. Uterus not described. Left ovary not described further.

Bergstrand,⁶ Case 6

Age. 22 years.

Menstrual History. Had never menstruated.

Masculinization. Hirsutism was present.

Postoperative Condition. Alive 9 years after operation. Hirsutism less, but had not entirely disappeared.

Ovarian Tumor. Right, mucinous cystadenoma size of man's head, lined by cylindrical mucoid epithelium. At one spot there was a dense accumulation of cells resembling lymphocytes and macrophages, very similar to case 5 (seminoma-like). Hyaline bodies similar to corpora atretica, stroma similar to that of ovary.

Felicissimo Paula Xavier and de Abreu Junqueira,¹² Single Case

Age. 34 years.

Menstrual History. Began at 14 years, more or less regular. Married for 15 years. Positive Wassermann test.

Pregnancies. Pain in abdomen with pregnancy, nausea of pregnancy. Tumor discovered at 5 months. Three children, four pregnancies; final pregnancy complicated by tumor.

Masculinization. Masculine habitus with male distribution of hair.

Postoperative Condition. Two months after removal of ovarian tumor stated that hair on face was growing rapidly and voice was worse. Was changed in appearance, with gross, acromegalic features, low harsh voice, beard, hypertrophy of larynx. Forearms hairy and rough. Acne pustules on face, back and chest, clitoris hypertrophied. Painful tumor in left iliac fossa. Fetus active. A verrucous nevus on anterior abdominal wall had become hypertrophied and hairy. Albuminuria and hypertension with edema. Premature rupture of membranes, fetal death, craniotomy. Fetus was female, masculinized, with hypospadiac clitoris. Maternal death.

Ovarian Tumor. Right, firm, solid, encapsulated, 9 by 5.5 by 4 cm. Some parts resembled adenoma tubulare, some with lumina, some solid cords. Cells in lymphatics of the pedicle. Corresponds to Meyer's arrhenoblastoma, group 2 (mixed type).

Uterus and Adnexa. Not described.

Plate,² Single Case

Age. 26 years.

Menstrual History. Began at 14 years, regular until 2 years and 9 months before, when periods of amenorrhea occurred lasting 4 to 5 months, ending in a hemorrhage longer and more severe than a normal menstrual period. Prior to admission there had been amenorrhea for 11 months. Married but never pregnant.

Masculinization. When the menstrual disturbances began, a growth of hair appeared upon the face, and the voice became deeper. Physical examination revealed that the mammae were well developed. Hair on abdomen was of masculine type. The voice was conspicuously deep. Clitoris and vagina were normal in size and consistence. The uterus was displaced to the right by a firm lump in the left half of the pelvis.

Postoperative Condition. Recovery. Menstruated normally 1 month after operation. The amount of hair on the face diminished but remained unchanged on the abdomen. The voice was deep.

Ovarian Tumor. Left, yellow on section. Cavity filled with a coagulated mass of clear yellow color. A structure the size of a cherry on one pole resembled a corpus luteum. Between the cavity and this structure were islets of intense yellow color. Microscopically, strands and islets resembling granulosa cells in folliculoid arrangement merged with tubules. Tubules were lined with low cylindrical epithelium. The cavity was lined with granulosa cells. The adenomatous portion corresponded with the patches of intense yellow color seen grossly.

Uterus and Adnexa. Curettage taken 7 days after operation showed practically normal endometrium.

If biological evidence of retained estrogenic activity plus masculinization is used as the criterion, not all of these cases fit into the category of gynandroblastoma. Histological evidence is not well enough defined to enable us to depend upon it for classification. Meyer's ¹ cases 6 and 7 meet the requirement. The three cases of Tietze ⁷ all show evidence of estrogenic activity by the tumors, in spite of the fact that in case 10 there was amenorrhea for 10 years, but evidence of masculinization is not recorded. All three tumors contained heterogeneous cell types. Amati's ⁸ case also lacked signs of masculinization, although there was complete amenorrhea for 5 years, and the tumor in part resembled testicular adenoma tubulare. In all three of Schiller's ⁹ cases menstruation was normally established, then became irregular with final amenorrhea. Masculinization was not complete. All three tumors were cystic and more nearly resembled granulosa cell tumors than arrhenoblastomas.

In Eerland and Vos' ¹⁰ case 2 the patient was amenorrheic, showed masculinization and began to menstruate 5 weeks after operation. Here the case history is that of arrhenoblastoma, but the tumor was not to be distinguished histologically from granulosa cell tumor. In their case 5, the patient was amenorrheic, with a beard. Menstruation returned soon after operation and growth of hair on the face disappeared. The tumor was composite and if the biological evidence of amenorrhea is omitted it may be considered as a gynandroblastoma. Their case 13 lacks sufficient data for analysis.

Frankl's ¹¹ case appears to belong in this category, although the menstrual history was not given.

Bergstrand's ⁶ cases, while not included by Plate, ² seem to be pertinent to the problem. In all five there was hirsutism. In case 1 the menses ceased at the time hirsutism appeared. In cases 2 and 3 the menstrual history was not stated. In case 4 the menses ceased 2 years before hirsutism appeared. In cases 5 and 6 the patients had never menstruated. In case 1 there were bilateral cystic tumors with structures resembling corpus luteum and granulosa cells. In cases 2 and 3 the tumors were partly tubular with interspersed lipid-containing

cells. These tumors answer the description of arrhenoblastomas. Case 4 was a complex tumor containing mucus-producing epithelium together with two other cell types, one of which was large and rich in cytoplasm with large round nuclei; the other was small, dark, poor in cytoplasm and formed garlands. In case 5 tumors were bilateral, larger on the right, and in part the structure suggested seminoma. These cases are suggestive of dysgerminoma, but with masculinizing effect due to an unidentified androgenic component. Case 6 is a cystic tumor with characteristics of pseudomucinous cystadenoma but with accumulations of cells of lymphocytic and macrophage type, similar to those in case 5.

Plate's² case is significant clinically and presents a complex histological structure consistent with both granulosa cell tumor and arrhenoblastoma. Through the courtesy of Dr. Walter Schiller, Cook County Hospital, Chicago, we have had an opportunity to study sections of this tumor. Interstitial cells in small groups were present but were not numerous. Lipoid storage was not prominent in any of the cellular elements. Mucus-secreting epithelium was not found in the sections available.

Schiller¹³ accepted only three cases in Plate's² series as gynandroblastomas. These were Plate's, Frankl's, and Schiller's case 4. Schiller¹⁴ has published a detailed description of his case, interpreting it as a gynandroblastoma. In addition to these three and the present case, Schiller cited the unpublished case of Christopoulos for inclusion in this group.

The case of Felicissimo Paula Xavier and de Abreu Junqueira,¹² while classified as arrhenoblastoma, is included because it illustrates androgenic effects in pregnancy not previously demonstrated in women but paralleling those produced experimentally in animals (Greene, Burrill and Ivy¹⁵). Developing during the course of pregnancy, the tumor was removed at 5 months but recurred within 2 months and resulted in masculinization of the mother and of the female fetus, which was delivered instrumentally near term.

COMMENT

Ovarian tumors exist in which there is alteration of the secondary sexual characters of the bearer in the male direction, presumably as a result of the androgenic hormone liberated by the tumor; but at the same time there is a continuation of cyclic menstrual bleeding, with indications of hyperestrinism. Such cases differ from granulosa cell tumors both biologically and histologically. Robert Meyer¹ first applied the term gynandroblastoma to them. There is no constant cell

pattern in these cases, and they appear to represent combinations of granulosa cell tumors and arrhenoblastomas. The presence of tubular structures lined by cylindrical, mucus-secreting epithelium has been noted in these, as well as in arrhenoblastomas and in the seminoma-like tumors of Bergstrand.⁶ Interstitial cells carrying lipoids, resembling the testicular Leydig cells, are common to both gynandroblastomas and arrhenoblastomas. There is presumptive evidence that these cells are the source of the androgenic hormone.

The question of antagonism between androgenic and estrogenic hormones in gynandroblastomas is complex. Androgenic hormones are capable of inhibiting menstruation in the normal woman, theoretically by inhibiting the gonadotrophic factor of the hypophysis, thereby preventing ovarian formation of estrin. This does not apply in case of the tumor, necessarily, as the tumor cells may be able to produce estrin independently of hypophyseal factors. Therefore, hyperestrinism and virilism may co-exist in the same person. It is possible also that hormones produced by tumors may differ from those originating normally in the gonads after puberty, both chemically and in their physiological effects. Frankl¹¹ mentioned Halban's theory that persons who become masculinized possess a latent intersexuality necessary to respond to the presence of androgenic hormone. Clinical experience with therapeutically administered androgenic hormones, as reported by Geist, Salmon, Gaines and Walter,¹⁶ and by Greenhill and Freed,¹⁷ does not prove the theory of a restricted latent intersexuality, but indicates that latent intersexuality, in the sense of possession of receptors for the androgenic hormones, if not universal must be very common. From the work of Geist and co-authors it appears that doses of less than 200 mg. of testosterone propionate do not suppress menstruation or cause any demonstrable changes in endometrium or vaginal smears, while larger doses (more than 500 mg. per month) produce some or all of the following effects: temporary amenorrhea, senile vaginitis, hoarseness, hirsuties, acne and enlargement of the clitoris.

Bergstrand⁶ rejected all earlier theories, ascribing hirsutism to the internal secretion of tumors arising in an hypothetical testicular component of the embryonic ovary. He found no indication from his studies whether the hormone is produced by the granulosa or the lutein cells of the tumors, but subscribed to the theory of Steinach and Kun that the hormone comes from the lutein cells. At least two and possibly three of his cases are of great interest in that combinations of androgenic components with dysgerminoma are suggested by the descriptions. Sailer¹⁸ has described granulosa cell nests in ovarian dysgerminoma. Ewing¹⁹ has presented evidence that embryonal carcinoma of

the testicle is of teratomatous origin and L'Esperance²⁰ has shown the analogy between this and embryonal carcinoma of the ovary. The fact that testicular tumors of this type produce gonadotrophic hormone was first observed by Zondek²¹ and has since been extensively studied by Ferguson²² and further amplified by Twombly, Temple and Dean,²³ who found little correlation between the amount of gonadotrophic hormone in the urine and the histological type of the tumor. It may be considered, however, a strong indication of the teratomatous nature of such tumors.

The opinion of Krock and Wolferman²⁴ that arrhenoblastomas do not constitute a pathological entity but represent teratomas containing virilizing elements is borne out equally well in gynandroblastomas, in which histological structure is also variable.

CONCLUSION

The term gynandroblastoma may be usefully applied to describe a clinical-pathological syndrome, but there is no constant accompanying histological pattern. The ovarian tumor concerned has epithelium-lined tubules and interstitial cell groups in common with arrhenoblastoma. The impression is gained that combinations of granulosa cell tumor with arrhenoblastoma do occur but that one element or the other usually predominates to the extent that the double biological effect is lacking. It is suggested also that the gynandroblastomas are teratomatous.

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[Illustrations follow]

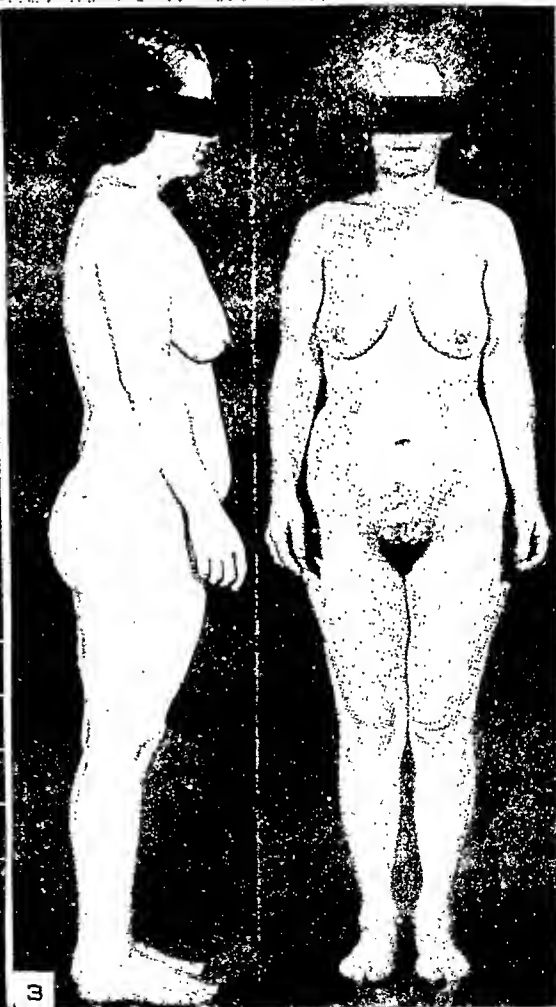
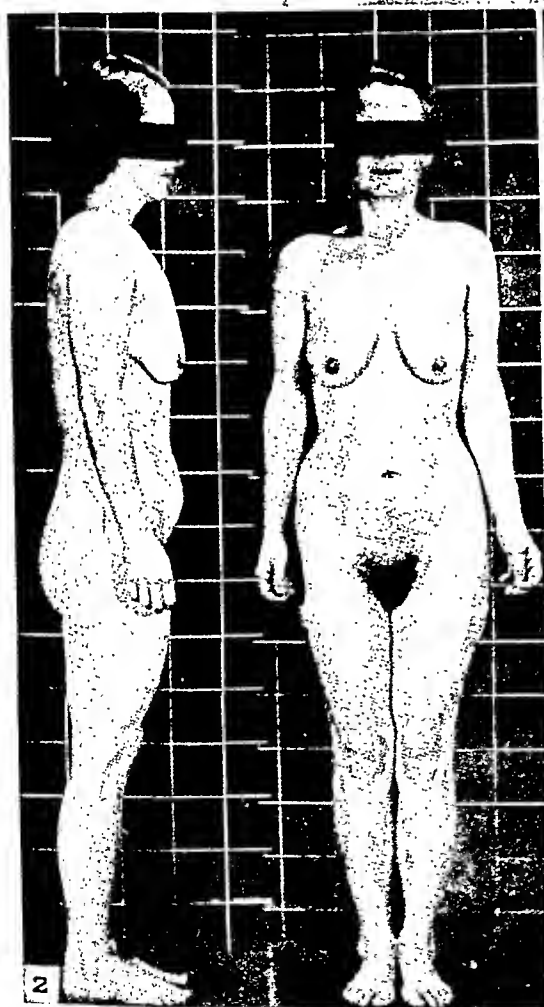
DESCRIPTION OF PLATES

PLATE 73

FIG. 1. Growth of beard and mustache 3 days after shaving.

FIG. 2. Physical appearance of the patient before operation, with hirsutism, acne vulgaris, a large larynx and pendulous breasts. The patient had shaved shortly before these pictures were taken.

FIG. 3. Gain in weight and reduction in hirsutism 6 months after operation.



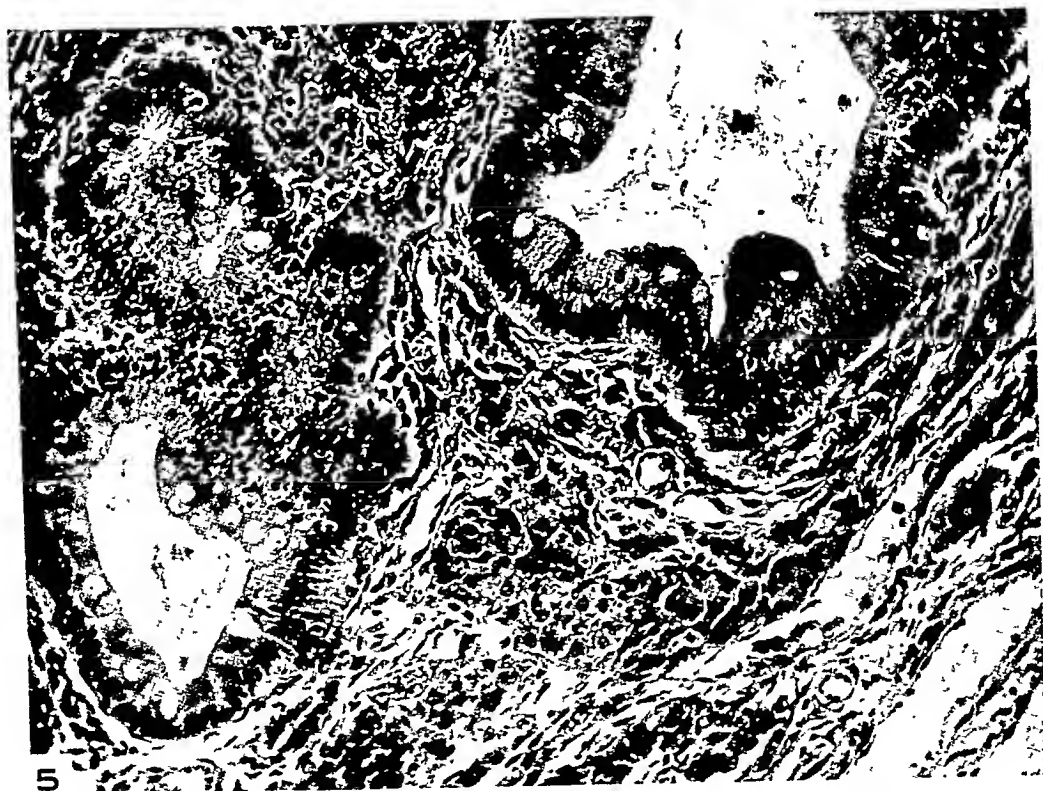
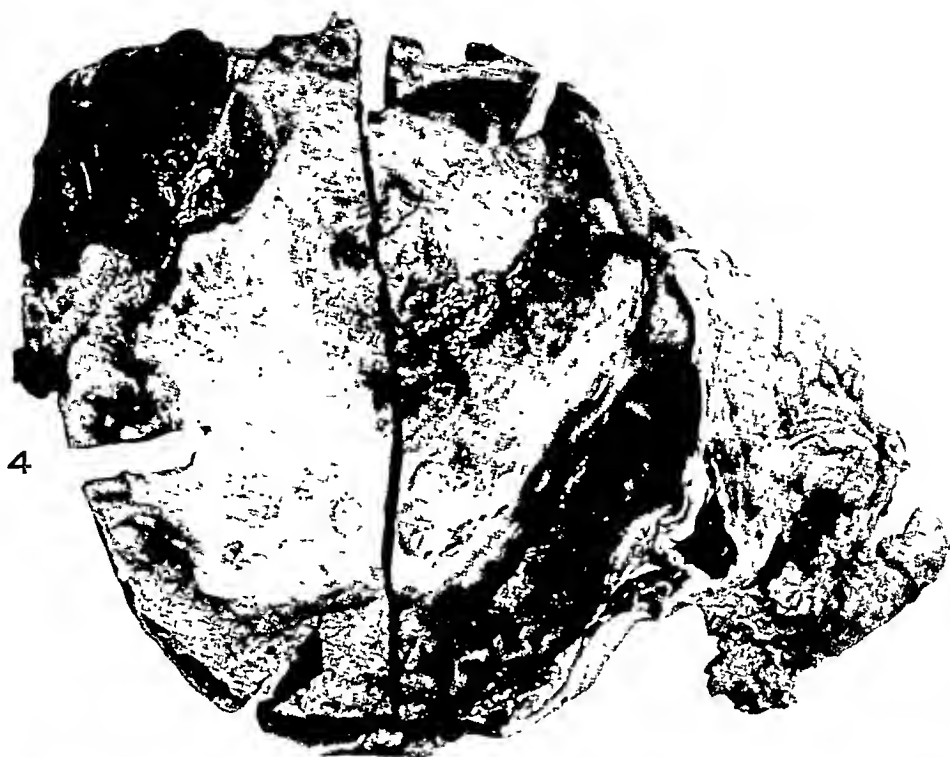
Mechler and Black

Gynandroblastoma of the Ovary

PLATE 74

FIG. 4. The cut surface of the tumor and ovary.

FIG. 5. Epithelium-lined tubules and interstitial cell groups in the tumor. $\times 150$.



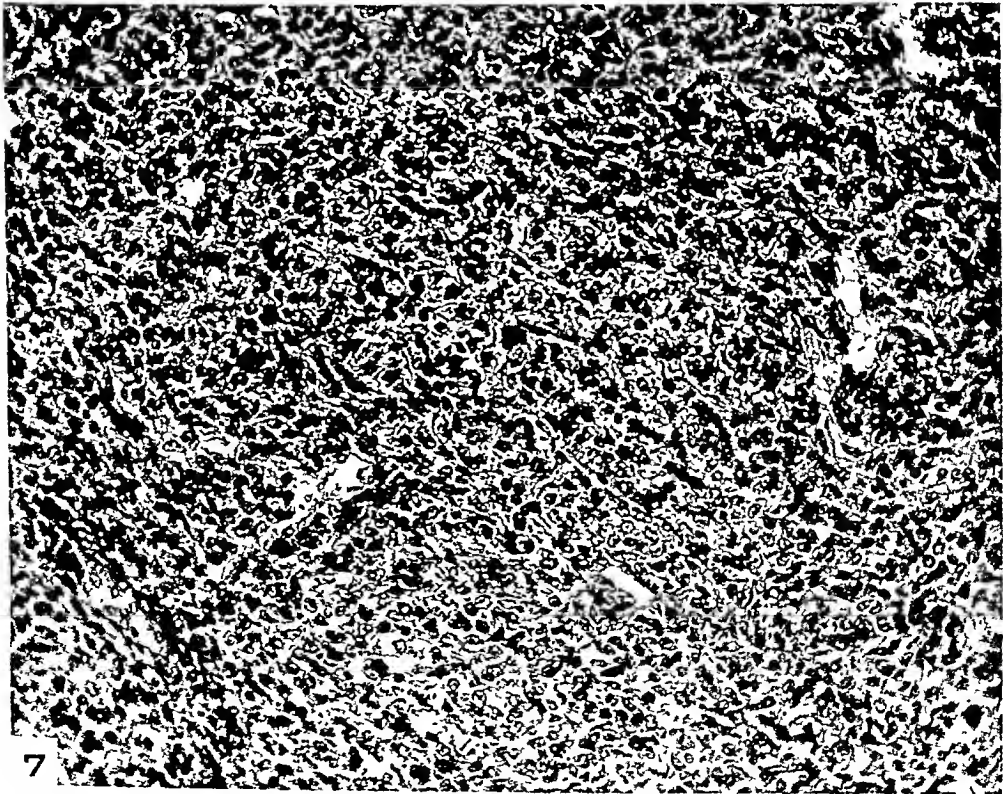
Mechler and Black

Gynandroblastoma of the Ovary

PLATE 75

FIG. 6. Lobular forms with granulosa cell pattern at the bottom of the field and solid cords and whorls at the top. There is a central island of imperfect plicated tubules. $\times 75$.

FIG. 7. A lobule of the tumor of granulosa cell type. $\times 150$.



STUDY OF SENSORY GANGLIA IN MACACA MULATTA AFTER GASTROINTESTINAL ADMINISTRATION OF POLIOMYELITIS VIRUS *

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In 1910, Leiner and von Wiesner ¹ reported success in infecting the rhesus monkey with the virus of anterior poliomyelitis when it was administered by feeding. Since that time other attempts of a similar nature have been made with largely negative or equivocal results.²⁻¹⁰ The virus has been fed, and given by stomach tube and by rectal tube, often with the introduction of accessory factors designed to improve the chances for infection; but experimental anterior poliomyelitis with paralysis and typical changes in the spinal cord has rarely resulted. The virus has, on occasion, been recovered from the feces of monkeys during a period when it was being fed intensively.^{8, 9} In spite of this fact it is not clear from the literature whether in the rhesus monkey the barrier against infection via the gastrointestinal tract lies in the lumen of the tract or in its mucosa and wall. However, if the virus can survive at all in the lumen, it is conceivable that some agent or agents exist which might carry it across the mucosa and into contact with the nervous system.

In a previous report ¹¹ the occurrence of certain lesions of the peripheral nervous system in experimental poliomyelitis is described. These lesions were present in the sensory ganglia of monkeys following intraperitoneal inoculation of human stool. No accompanying changes were observed in the central nervous system. When, therefore, experiments were planned to attempt to break the intestinal barrier against poliomyelitis virus, it was borne in mind that the end-point of the experiment might not be clinical paralysis with destruction of the anterior horn cell, but rather subclinical disease with some evidence of involvement in sensory ganglia.

GENERAL TECHNIC

Macaca mulatta were used; in the test group, animals weighing between 2200 and 2800 gm. were selected. No selection was practiced regarding sex. The period between the final administration of virus and sacrifice of the animals varied and is explained under each experiment. All sacrificed animals, both test and control, were killed by intracardiac injection of air and were autopsied immediately.

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In examining the central nervous system at least one block of tissue was taken from the sacral, one from the lumbar, three from the dorsal and one from the cervical region of the spinal cord. Three blocks were taken from the medulla oblongata, one from the mesencephalon, and three from the diencephalon. The paravertebral sympathetic chain from the stellate ganglion to the brim of the pelvis was taken from each side. Blocks of tissue were taken from each major division of the cerebral cortex and from the cerebellum.* Following the description of Howell and Straus¹² of the intervertebral ganglia, an attempt was made to recover seven pairs of cervical, twelve pairs of thoracic, seven pairs of lumbar, three pairs of sacral, but only three pairs of caudal ganglia; search for the last caudal pair was omitted. The ganglia, regarding segment and side, were identified throughout the subsequent procedures. Two vagal and two gasserian ganglia and both olfactory bulbs were recovered but not identified as to side.

All tissues were fixed immediately in 70 per cent ethyl alcohol, blocked in paraffin, cut at 10 μ , and stained with thionin. Obviously, the examination of the central nervous system was on a basis of sampling, but the peripheral ganglia and olfactory bulbs were cut serially and thoroughly scrutinized. Since the smallest lesions found extended through only three to five paraffin sections, an attempt was made never to lose more than two or three consecutive sections from any ribbon, but this aim could not always be achieved. The actual number of sections of spinal ganglia obtained from each animal is given in Table I. The examination of both test animals and controls was equally extensive and the observations are therefore believed to be comparable. In the actual microscopic study every second section was inspected and if suggestive changes were seen, adjacent sections were also examined (Figs. 1 to 16).

THE LESIONS

In severe experimental poliomyelitis in monkeys, lesions of the spinal ganglia are prominent and easily identified. Flexner and Lewis⁴ described the main attributes, but they dealt principally with ganglia in which widespread destruction of nerve cells with marked inflammatory reaction was present. Early in the current investigations it became apparent that no such striking tissue alterations could be found. Inflammatory foci were rare and of minute proportions, usually involving, at the most, one or two neurons in an entire ganglionic mass and only

* Although changes were noted in the sympathetic ganglia, a description of these changes or of the tissues taken from the cerebral cortex where lesions were not observed is not given, since the examination could not be made in sufficient detail to warrant any conclusions being drawn.

occasionally as many as four or five neurons. Perivascular reactions were for the most part lacking and the interstitial spread of inflammatory cells was usually confined to the immediate vicinity of the nerve cell. Since cytologic alterations in neurons were not thought to be safe guides, it was often difficult to be certain that in a focal lesion a neuron had actually been destroyed. The inflammatory or non-nervous cells in a reaction were largely lymphocytic types, occasional polymorphonuclear cells were seen, and some cells resembling plasma cells were prominent, especially in the vagal ganglia. Sometimes, too, cells that might have been swollen pleomorphic forms of capsular cells seemed to dominate the picture. It is not intended to define here the exact identity of these cells, only to indicate that most of them were of mononuclear type.

If it is assumed that when the virus of poliomyelitis attacks the nervous system it acts primarily by destroying or altering neurons, then the smallest unit of pathologic change must be that accompanying the involvement of a single neuron. On this basis, and since no specific diagnostic sign is available, it was decided that in counting lesions, any cellular focus localized about a disintegrated or partially disintegrated nerve cell, or the site where a nerve cell might reasonably have been, must be considered as a lesion. This criterion has been held in examining all animals, both those exposed to the virus and those in which no known exposure had occurred. In Tables I to III no attempt is made to gauge the severity of lesions; only their presence or absence is indicated.

EXPERIMENTAL PROCEDURE

Demme,⁷ in some of his experiments, had used saponin to prepare the gastrointestinal tract for the virus. In a previous study¹³ I have shown that Duponol,* a wetting agent, could be used in the presence of poliomyelitis virus to prepare stool specimens for intraperitoneal inoculation. It was hypothesized that because of its wetting properties such an agent might bring virus suspensions into more intimate contact with the intestinal mucosa and so facilitate the passage of the virus. If any of the lesions found during the first three experiments were due to the virus of poliomyelitis, there was little evidence that Duponol had altered the mechanism of infection.

First Experiment

A fresh passage of the Knox strain of poliomyelitis virus recovered from human feces¹⁴ was made intracranially into an anesthetized monkey. This monkey was sacrificed as soon as severe paralysis appeared; the spinal cord and brain stem were

* Duponol W. A. (flakes), a sodium lauryl sulfate, manufactured by E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.

TABLE I
Lesions in the Peripheral Nervous System of Rhesus Monkeys after Gastric or Rectal Administration of Poliomyelitis Virus*

Monkey no.	Inoculation	No. of spinal ganglia		No. of spinal ganglion sections seen	X nerve ganglia		V nerve ganglia		Olfactory bulbs		Clinical picture†
		Examined	With lesions		1	2	1	2	1	2	
245M	5% virus, 0.6% Duponol; 3 feedings, 25 ml.; regurgitated once	64	60		—	—	+	—	+	—	Poliomyelitis; killed 11th day
235M	5% virus, 0.6% Duponol; 3 feedings, 25 ml.; vomited once	58	11	4,034	—	—	+	—	—	—	Fever(?); killed 13th day
230M	5% virus; 3 feedings, 25 ml.	64	8	4,184	—	—	—	—	—	—	Uneventful; killed 20th day
248M	2% virus, 0.5% Duponol; 3 enemas, 115 ml.	63	1	3,697	—	—	—	—	—	—	Uneventful; killed 19th day
250F	2% virus, 0.5% Duponol; 3 enemas, 110 ml.	64	8	2,308	+	—	—	—	—	—	Fever(?); killed 16th day
174M	2% virus; 3 enemas, 100 ml.	64	16	3,481	+	—	+	—	—	—	Fever, diarrhea, tuberculosis; killed 19th day
285M	5% virus, 0.5% Duponol; 3 enemas, 150 ml.	64	8	4,996	—	—	—	—	—	—	Uneventful; killed 12th day
277M	5% virus; 3 enemas, 150 ml.	64	7	5,067	+	—	+	—	—	—	Uneventful; killed 12th day
322M	6% virus; 1 enema, 45 ml.; 1.5 mg. histamine, intravenously	64	13	6,798	+	—	—	—	—	—	Uneventful; killed 17th day
330M	6% virus; 2 enemas, 90 ml.; 1.5 mg. histamine, intravenously; 2.0 mg. histamine, intravenously	64	18	7,528	+	+	+	—	—	—	Uneventful; killed 21st day
328F	6% virus; 2 enemas, 90 ml.	64	9	7,013	+	—	+	+	—	—	Uneventful; killed 21st day

First experiment:
Knox strain, by stomach tube

Second experiment:
Rockefeller M.V. strain, by rectal tube

Third experiment:
Rockefeller M.V. strain, by rectal tube

Fourth experiment:
Rockefeller M.V. strain, by rectal tube

Rocekeller M.V. strain, by stomach tube	323M	20% virus; 1 feeding, 15 ml.; 2.0 mg. histamine, intravenously	63	7	6,700	—	—	—	—	—	—	Uneventful; killed 16th day
	326M	20% virus; 2 feedings, 30 ml.; 1.5 mg. histamine, intravenously; 2.0 mg. histamine, intravenously	64	9	5,225	—	+	+	—	—	—	Uneventful; killed 21st day
	325L	20% virus; 2 feedings, 30 ml.	64	3	6,104	+	+	—	—	—	—	Questionable; killed 22nd day
Control animals: Monkeys having no inoculation, sacrificed or dying of conditions other than poliomyelitis	292	None	64	—	3,752	—	—	—	—	—	—	Died; enteritis, <i>B. dysenteriae</i>
	293	None	57	1	4,205	+	—	—	—	—	—	Died; pneumonia
	331	None	63	—	4,596	—	—	—	—	—	—	Sacrificed; tuberculosis, <i>B. dysenteriae</i>
	391	None	64	2	7,968	+	—	—	—	—	—	Sacrificed; emaciated
	392	None	64	4	6,885	—	—	+	—	—	—	Sacrificed; tuberculosis
	394	None	64	2	9,015	—	—	—	—	—	—	Sacrificed; ill, emaciated
	395	None	64	—	8,237	—	—	—	—	—	—	Died; cause undetermined
	396	None	61	—	7,000	—	—	—	—	—	—	Sacrificed; ill

* The central nervous tissues and olfactory bulbs of all, excepting monkey 245, failed to show changes and hence are not listed in the tables.

† All times are figured from the date of first inoculation.

+ = Any lesion of whatever severity.

— = Structure was examined and no lesion seen.

A blank space indicates that the structure was not examined.

ground with sterile alundum and taken up in sterile distilled water to make a 5 per cent virus-cord suspension. The suspension was divided into three equal portions. To two of them Duponol was added in 0.6 per cent concentration. All three portions were stored at 4° to 8° C. when not in use.

Three monkeys were chosen and the suspensions were introduced by gastric tube on 3 successive days beginning on September 10, 1941, each animal receiving a total of 25 ml. Monkeys 245 and 235 were given Duponol-treated suspension; monkey 230, nontreated suspension.

On the first administration, monkey 245 regurgitated part of the feeding and in 7 days showed typical clinical signs of poliomyelitis with fever and paralysis. The classical lesions of experimental poliomyelitis were found in its spinal cord, brain stem, sixty of the spinal ganglia, one gasserian ganglion, and one olfactory bulb. Because of olfactory bulb involvement it must be assumed that the portal of entry was by way of the olfactory mucosa. This animal therefore is excluded from the percentage derivatives for sensory ganglia found in Table III.

Monkey 235 had a slight increase in temperature on the eighth day but showed no other signs of disease. Lesions were found in eleven spinal ganglia and in one fifth nerve ganglion.

Monkey 230 had an uneventful clinical course; eight spinal ganglia showed lesions.

Second Experiment

A month later, in October, 1941, a 2 per cent suspension in physiologic saline solution of a freshly passed virus of Rockefeller M. V. strain was divided as before into three parts. One part was left untreated and to the others two parts Duponol was added to make a 0.5 per cent concentration. Three monkeys were chosen and this time the virus suspensions were administered by a rubber tube, 6 to 8 inches of which was passed into the rectum. (See Tables I and II.)

Monkey 250 received on 3 successive days rectal inoculations totaling 110 ml. of Duponol-treated suspension. It showed questionable fever on the fifth and sixth days but no other clinical signs. Eight spinal and one vagal ganglia were involved.

Monkey 248 also received on 3 successive days inoculations totaling 115 ml. of Duponol-treated suspension and had an uneventful clinical course. A single minimal lesion was found in one spinal ganglion.

Monkey 174 was given on 3 successive days inoculations totaling about 100 ml. of untreated virus suspension. It had marked fever with some diarrhea on the 13th and 14th days and subnormal temperature on the 16th day. At autopsy mild tuberculosis was found. Sixteen spinal ganglia, one vagal ganglion, and one gasserian ganglion showed focal lesions.

Third Experiment

In December, 1941, after a number of control animals had been examined and it was apparent that some difference existed between them and the test animals, a third experiment was set up. The M. V. strain was freshly passed and the infected cord ground as before. The suspension this time was made in sterile distilled water to a 5 per cent concentration and divided into two portions. To one, Duponol up to 0.5 per cent was added; the other was untreated. Two monkeys were chosen and each received a rectal inoculation on 3 successive days. The total was 150 ml. per animal.

Monkey 285 was given the Duponol-treated suspension; it had an uneventful clinical course and eventually showed lesions in eight spinal ganglia.

Monkey 277 received untreated virus suspension. It showed no clinical signs of poliomyelitis and had involvement of one vagal and one gasserian ganglion, in addition to lesions in seven spinal ganglia.

TABLE II
Distribution by Segment and Side of Lesions in the Intervertebral Ganglia

Monkey no.	Cervical								Dorsal								Lumbar							Sacral			Caudal			
	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	1	2	3	4
235 L R	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
230 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
323 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
326 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
325 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
248 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
174 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
285 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
277 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
322 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
330 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
328 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
292 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
331 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
293 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
392 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
394 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
391 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
395 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
396 L R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = Any lesion of whatever severity.

- = Structure was examined and no lesion seen.

A blank space indicates that the structure was not examined.

The frequency and distribution of lesions as given in the tables and the quality of the lesions as illustrated give some idea of the problem as it existed after the third experiment. In the tissue examinations the animals had not been treated as unknowns; that is, the examiner was aware which were test and which control animals. As a check, therefore, the sections from five test animals were selected by an assistant. These were re-examined as unknowns and with the exception of a single instance, the lesions were reidentified accurately.

Since it was conceived that the changes in the intestinal tract induced ¹⁵ by histamine shock might alter its barrier against the virus, a final experiment was planned in May, 1942, to test this idea, and to check previous experiments.

Fourth Experiment

Along with the examination of more control animals, Rockefeller M.V. strain was freshly passed as before, this time in two animals. After quadriplegia occurred they were sacrificed, histologic examinations were made for characteristic cord changes, and the cords and brain stems ground and suspended in sterile distilled water. The initial strength of this suspension was 20 per cent. It was divided in two parts. One part contained enough for five doses of 15 ml. The other part was diluted with sterile distilled water to make a 6 per cent suspension. The volume was sufficient for five doses of 45 ml. each.

Three monkeys were selected for gastric inoculation, three for rectal inoculation. It was known that the action of histamine might induce vomiting and defecation. The procedure of inoculation had therefore to be accommodated to these facts. In gastric inoculation food was withheld for 18 to 24 hours before intubation. After the introduction of the virus, the animals were hosed with tepid water and isolated. Three hours later histamine dihydrochloride was given intravenously and the monkey quickly placed in a separate cage until the reaction had ceased. It was then hosed thoroughly again and returned to its quarters. The cage where the reaction occurred was well cleaned. With this method, although retching occurred, no monkey vomited more than a small quantity of mucus.

The rectal inoculations were conducted similarly, although the interval between intubation and the exhibition of histamine was reduced to one-half hour. The interval was based on an estimate of the time required for spreading the inoculum over the lower bowel.

The histamine used was a commercial preparation of a 1:1000 solution of histamine dihydrochloride. In none of the animals inoculated was a severe reaction induced.

Monkey 322 was given one enema, 45 ml. of virus suspension, and one injection of histamine. The clinical course was uneventful and lesions were found in thirteen spinal ganglia and one vagal ganglion.

Monkey 330 was given two virus enemas, a total of 90 ml., and two injections of histamine. There were no clinical signs. Eighteen spinal ganglia, both vagal ganglia, and one gasserian ganglion showed lesions.

Monkey 328 was given two virus enemas, a total of 90 ml., but no histamine. There were no clinical signs. Nine spinal ganglia, one vagal ganglion, and both gasserian ganglia showed some involvement.

Monkey 323 was given one stomach feeding of 15 ml. of virus suspension, and one injection of histamine. No clinical signs were observed. Seven spinal ganglia showed lesions.

Monkey 326 was given two stomach feedings totaling 30 ml. of virus and two histamine injections. The clinical course was uneventful. Nine spinal and both gasserian ganglia proved to be involved.

Monkey 325 was given two stomach feedings of virus, a total of 30 ml., but no histamine. Questionable fever occurred. Examination of the nervous system showed three spinal and both vagal ganglia to be involved.

Lesions of the spinal ganglia were more frequent in animals receiving histamine; those given two inoculations, both of virus and histamine, had more lesions than those receiving one. The difference, however, is not so clear when one compares vagal or gasserian ganglia. These data do not seem sufficient to allow any conclusions regarding the activity of histamine in breaking the barrier against virus.

Controls

Since apparently healthy monkeys were at a premium, sick animals were chosen. Most of them were killed in the usual manner when it became evident that they were seriously ill. A few died and were autopsied up to within 6 hours after death. Animals that were thought to have been dead for a longer period were discarded. Signs of disease, though not necessarily the cause of death, were recorded. It was believed, moreover, that this control group represented the commoner disease pictures found in laboratory colonies of rhesus monkeys, and that nonspecific lesions of the nervous system would be more evident than in healthier animals.

In many of the animals no lesions could be found and in the others they were sparse, a total of thirteen ganglia in eight animals showing involvement.

Tables I and II give the number and distribution of the focal lesions. Table II contrasts the difference in the distribution of lesions in animals receiving stomach feedings and those given the virus by rectum. It demonstrates, too, that the differences are in the number of ganglia involved, but not in the distribution of the involvement.

Table III summarizes numerically Tables I and II and gives the percentage of involvement of sensory ganglia. The brain stem and olfactory bulbs are omitted since no lesions were observed in these regions.

TABLE III
Summary of Findings in Sensory Ganglia

Type of inoculation	Spinal ganglia		X nerve ganglia		V nerve ganglia		Percentage of ganglia with lesions		
	No. examined	No. with lesions	No. examined	No. with lesions	No. examined	No. with lesions	Spinal	X nerve	V nerve
Stomach tube	313	38	10	2	8	3	12	20	37
Enema	511	80	16	7	16	5	16	44	31
Control	501	9	16	2	16	2	2	12	12

DISCUSSION

The lesions described are for the most part very minute and by any criteria known to me are nonspecific. The significance of the observations therefore rests entirely upon the thoroughness of examination and the accuracy of identification of the lesions in test animals and controls.

Since no series of animals inoculated with sterile monkey cord was examined, the possibility that pathologic change in sensory ganglia might be produced by such inoculations cannot be excluded. That possibility seemed so remote, however, that it was thought inadvisable to expend animals for the purpose of determining it.

If it be allowed that most of the nerve cell destruction observed in the test animals was produced by poliomyelitis virus, then several features brought out in the tables are of interest. Firstly, *Macaca mulatta* actually may be infected via the gastrointestinal route. In future one may be better able to examine the natural limitations imposed on the infectious agent by this portal, or, in other words, to inquire more exactly what and where is the barrier.

Secondly, the increased frequency of lesions of the spinal ganglia in the lower cervical, lumbar and sacral segments as compared with the upper cervical, dorsal and caudal segments is striking, especially since the segments most often involved correspond quite closely to the portions of the spinal cord functioning for the extremities. One is tempted to read into this situation some peculiar "hook-up" of the sensory system with the intestinal tract, until it is recalled that the lower cervical and lumbosacral ganglia are the largest in the intervertebral series and may therefore contain more nerve cells. In the face of this, any basic distributing system could make more contacts in these ganglia and hence give greater chance for involvement. Both of these ideas rest on the assumption that the virus travels from the gut to the ganglia centripetally in nerve processes. Actually, at this time no explanation is offered of the mechanism accounting for the occurrence or distribution of lesions in either spinal or cranial nerve ganglia. However, I am familiar with some of the work¹⁶ that describes the presence of unmyelinated fibers of the autonomic nervous system in the mucosa and submucosa of the bowel, and of unmyelinated nerve fibers, possibly sensory, ending in the epithelium itself.

Thirdly, when virus suspensions are put into the stomach of an animal, one might reasonably expect that if the agent survived it would eventually reach the rectum, having traversed the intervening tract. On the contrary, one would not expect virus put into the large bowel to reach the stomach or even most of the small intestine. Likewise, if in traversing the intestinal tract the virus particles were changed either as to quantity or infectivity, then one might anticipate slighter involvement from its inoculation into the stomach than into the colon. On examination of the tables of distribution for spinal ganglia, it is apparent that over the whole of either the feeding group or the enema group no marked difference in the pattern of segmental involvement can be

seen. But there is a difference of frequency between the two groups. The findings suggest that the virus may have to move along the length of the small intestine into the large intestine before passing the intestinal barrier and coming into effective contact with nerve cells. They also suggest that the virus may be, to some degree, altered or destroyed while resident in the stomach or small bowel.

Table III gives a comparison of the total number of lesions found in both groups of experimental animals and in the controls. The differences found in tenth and fifth nerve ganglia, while they are suggestive, are not statistically significant. On the other hand, a comparison of the percentage involvement of either the enema group or the feeding group with that of the controls demonstrates differences that are statistically significant.

SUMMARY

A count was made of all focal inflammatory lesions found in the vagal, gasserian, and intervertebral ganglia of a series of 14 monkeys to which poliomyelitis virus had been administered either by stomach tube or by rectal tube, and of those found in 8 monkeys not exposed to the virus. The difference in the percentages of intervertebral ganglia involved when test animals are compared with controls is statistically significant: 12 per cent in 6 animals fed by stomach tube; 16 per cent in 8 animals to which virus was administered by rectal tube; and 2 per cent in the control group of 8 animals.

Similar lesions were not found in the central nervous systems of the animals examined.

The distribution of focal lesions in the intervertebral ganglia follows a pattern throughout the test series. Lesions were strikingly more numerous in the lower cervical and lumbosacral groups of ganglia.

A comparison of the lesions in the intervertebral ganglia of monkeys given the virus by stomach tube with those in monkeys given the virus by rectum shows no difference in pattern, but the frequency of lesions in the former is suggestively less than that in the latter.

Treatment of virus suspensions with Duponol did not seem to increase the numbers of lesions found in sensory ganglia.

An attempt to facilitate the passage of virus from the intestinal lumen to the nervous system by administration of histamine dihydrochloride gave inconclusive results.

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[*Illustrations follow*]

9

DESCRIPTION OF PLATES

The plates are arranged so that lesions of similar severity in test and control animals may be readily compared. It should be noted that none of the lesions is extensive; those shown include the most severe, together with some less severe. Sections from control animals cover almost all the lesions found in that series.

Sections on the left in each plate are from sensory ganglia of monkeys exposed to the virus of poliomyelitis; those on the right are from monkeys that had no known exposure to the virus. Figure 15 shows a spinal ganglion from a monkey that had severe poliomyelitis with marked lesions of the spinal cord, and that was sacrificed during the acute stage of the disease. While the cellular reaction is not wholly similar to that shown in the other figures, it can be seen that the process involves the destruction of single neurons; in other words, that the total picture consists of many individual foci, any one of which may be compared with a focus in an experimental animal.

Tissues were fixed in 70 per cent alcohol, cut in paraffin, stained with thionin, differentiated in alcohol.

PLATE 76

FIG. 1. Monkey 322. Right 7th cervical ganglion. Virus administered by enema. Sacrificed on the 17th day. Figures 1 to 4 represent the more marked type of lesion found in test animals and show loss of one or more neurons, with fairly dense inflammatory reactions. $\times 125$.

FIG. 2. Monkey 328. Right 1st sacral ganglion. Virus by enema. Sacrificed on the 21st day. Section represents the severe lesion. $\times 125$.

FIG. 3. Monkey 277. Gasserian ganglion. Virus by enema. Sacrificed on the 12th day. Section represents the severe lesion. $\times 125$.

FIG. 4. Monkey 328. Left 2nd sacral ganglion. Virus by enema. Sacrificed on the 21st day. Section represents the severe lesion. $\times 125$.

FIG. 5. Monkey 394. Left 2nd lumbar ganglion. No virus. The animal was emaciated and was sacrificed; no specific disease was identified. Figures 5 to 8 represent more definite lesions in the control series. $\times 125$.

FIG. 6. Monkey 392. Left 2nd dorsal ganglion. No virus. When sacrificed, the animal exhibited extensive tuberculosis. Section represents more definite lesion in the control series. $\times 125$.

FIGS. 7 and 8. Monkey 293. Figure 7: Vagal ganglion. Figure 8: Right 8th cervical ganglion. No virus. Animal died spontaneously; pneumonia was found at autopsy. Sections represent more definite lesions in the control series. $\times 125$.

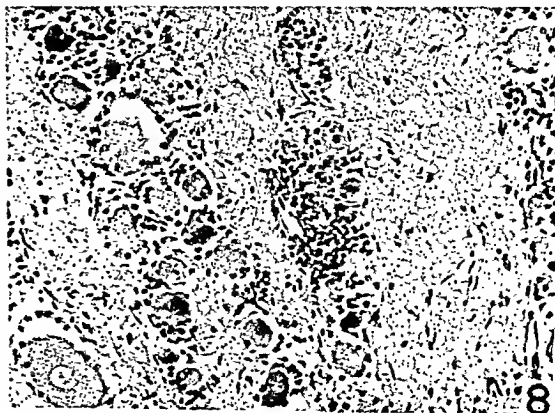
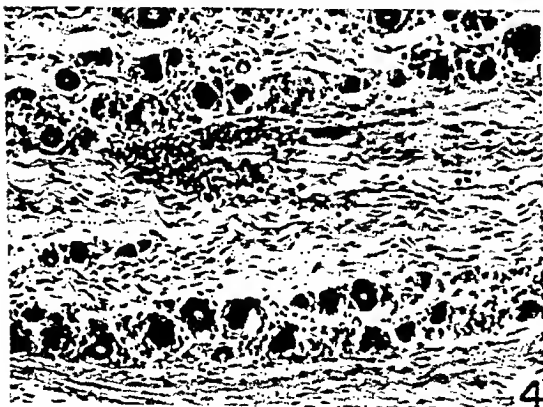
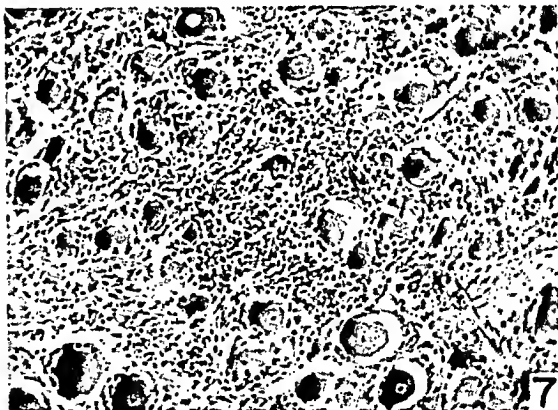
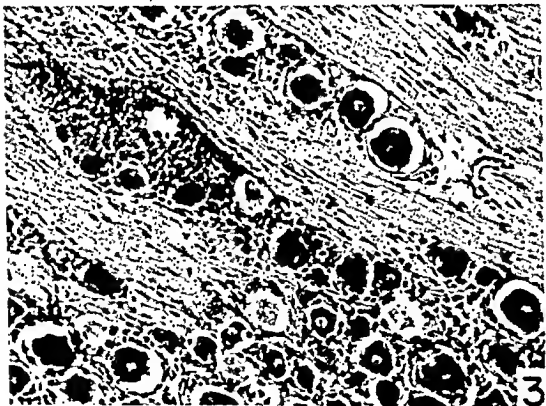
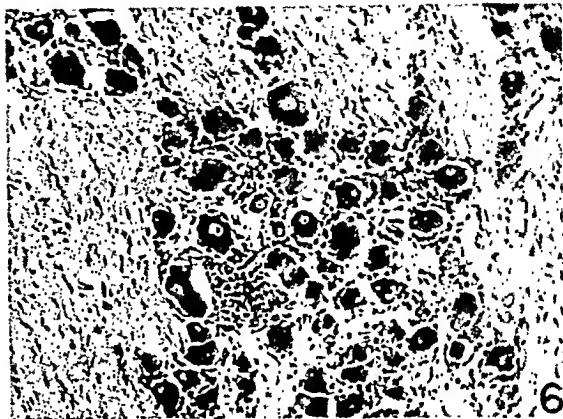
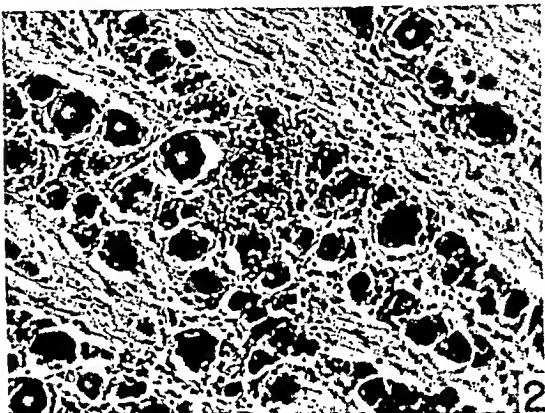
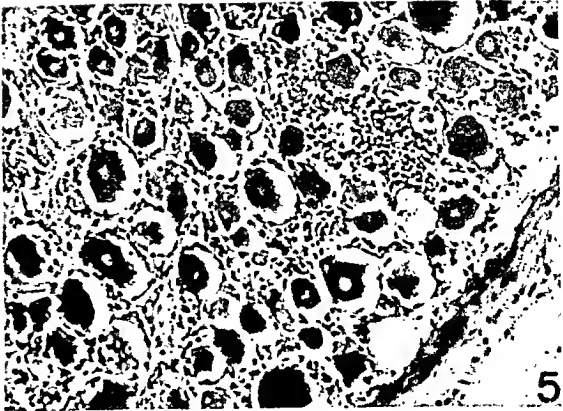
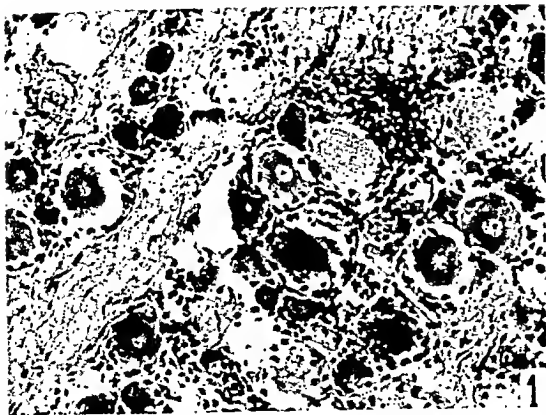


PLATE 77

FIGS. 9 and 10. Monkey 322. (See also Fig. 1.) Figure 9: Vagal ganglion. Figure 10: Left 2nd sacral ganglion. Virus administered by enema. Sacrificed on the 17th day. Figures 9 to 11 represent less severe lesions found in test animals. $\times 125$.

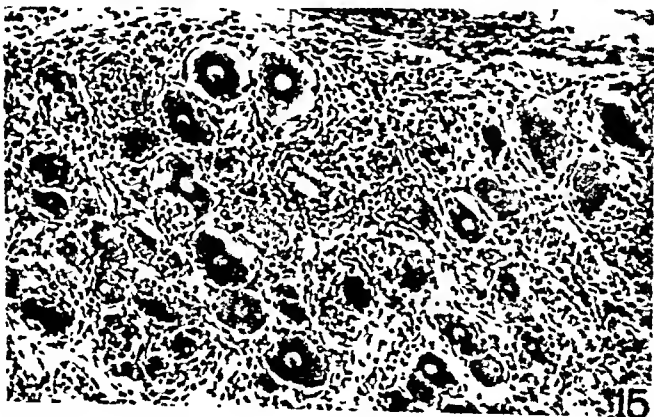
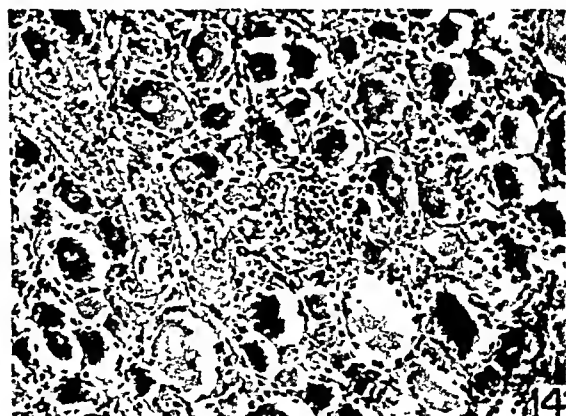
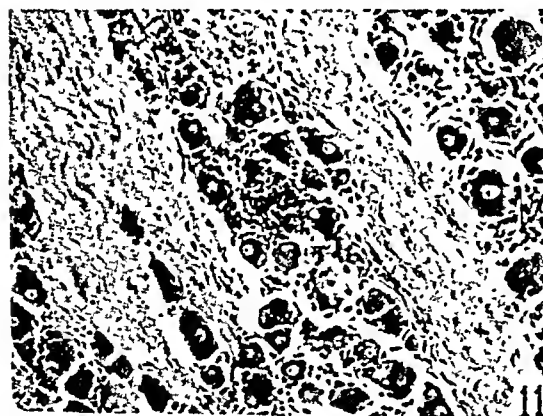
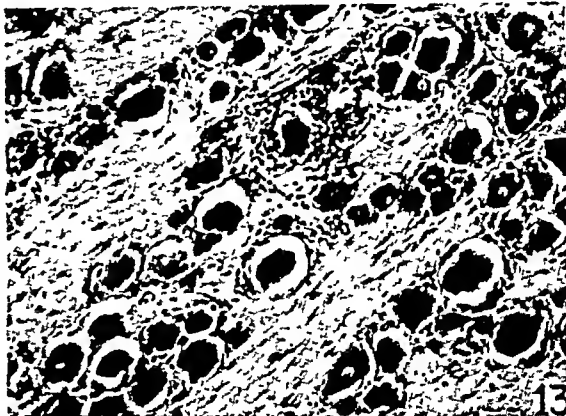
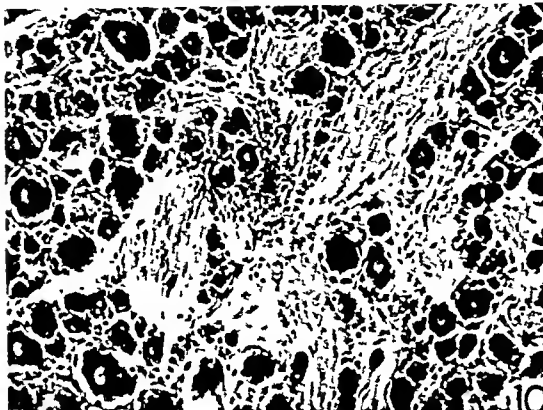
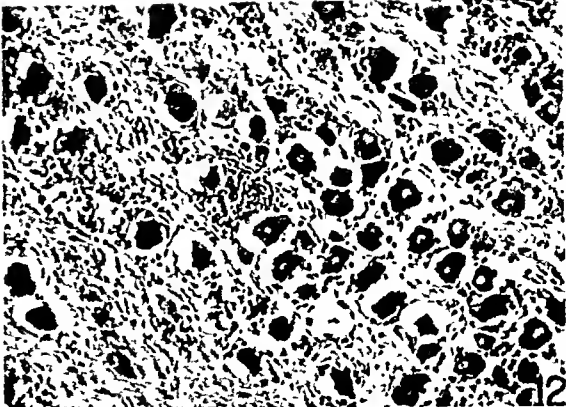
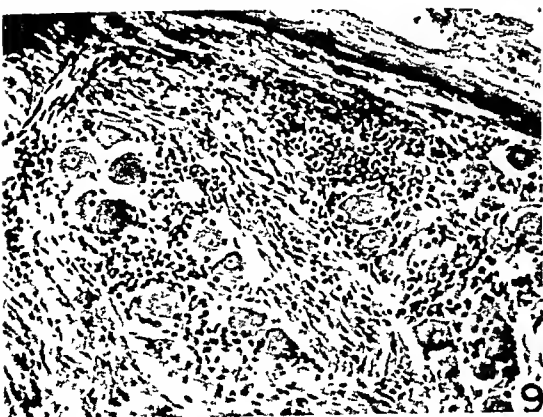
FIG. 11. Monkey 277. (See also Fig. 3.) Right 4th lumbar ganglion. Virus by enema. Sacrificed on the 12th day. Section shows less severe lesion found in test animals. $\times 125$.

FIG. 12. Monkey 391. Vagal ganglion. No virus was administered, but animal was sickly. It was sacrificed but no specific disease was found. Section shows less severe lesions found in control animals. $\times 125$.

FIG. 13. Monkey 392. (See also Fig. 6.) Gasserian ganglion. No virus. When sacrificed, animal exhibited extensive tuberculosis. Section shows less severe lesion found in control animals. $\times 125$.

FIG. 14. Monkey 394. (See also Fig. 5.) Left 5th lumbar ganglion. No virus. The animal was emaciated and was sacrificed; no specific disease was identified. Section shows less severe lesions found in control animals. $\times 125$.

FIG. 15. Intervertebral ganglion. Animal had severe poliomyelitis involving the spinal cord. It was sacrificed during the acute stage of the disease. $\times 125$.



THE PATHOLOGY OF CONVALESCENT POLIOMYELITIS IN MAN *

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The description of the pathology of poliomyelitis, like that of most acute and self-limited diseases, has been restricted almost entirely to the conditions obtaining at the climax of the illness. Death, when it occurs, may either result early from an overwhelming infection, or more commonly from respiratory failure at the crisis on or about the fifth day. Practically, these are the patients which the pathologist gets the opportunity to examine. Those who survive the crisis as a rule recover, though with a varying degree of residual paralysis. Relapse or a second attack is exceedingly rare. Such survivors often live out the usual span of life, and the few who have come to autopsy years after their illness show, as expected, only patches of atrophy and sclerosis to mark those areas most severely damaged in the acute illness.

Attempts to obtain information on the pathology of the convalescent stage of poliomyelitis indirectly through animal experimentation are also subject to severe limitation. In part this is due to the fact that certain species of monkeys are still the only animals known to be susceptible to the general run of poliomyelitis virus. A more serious obstacle to the experimental investigation is the circumstance that poliomyelitis is a much more highly fatal disease in monkeys than it is in man. It is relatively easy to produce poliomyelitis in monkeys by inoculation with any of the laboratory-adapted strains of virus, but the great majority of infected animals die at the height of their illness in spite of nursing care. For this reason a large number of animals must be lost in order to obtain a few in the convalescent stage. The considerable expense and labor required has apparently prevented the carrying out of any planned experiment along these lines. However, from the accumulated material of the Rockefeller Institute, Warburg¹ obtained the brains of 15 monkeys which had lived from 19 to 309 days after inoculation. From these she prepared a detailed description of the histology of the stage of repair of poliomyelitis in the monkey.

The introduction of the mechanical respirator about 13 years ago, and its subsequent increasingly widespread use, might have been expected to provide some human subjects for the study of the pathology of convalescent poliomyelitis. However, few, if any, studies of that

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character have been published. Probably the reason is that the respirator does not alter the basic nature of the poliomyelitic process. Artificial respiration is generally unable to prolong, to any degree, the life of the patient with severe bulbar involvement. On the other hand, many patients with paralysis of the muscles of respiration, saved by the respirator during their acute illness, later recover sufficient functional power to become more or less independent of the respirator. Such patients pass through convalescence to the stage of permanent arrest and scarring, and may live many years thereafter. Only occasionally is the balance between life and death so delicately poised that some small accident can upset it and cause the death of the patient during the early convalescent period. The three cases to be described are among the very few of this sort that have come to autopsy.

REPORTS OF CASES

The three subjects of this report were victims of the 1941 outbreak of poliomyelitis in Tennessee. Two had been patients at the Vanderbilt University Hospital. The third was examined at the Erlanger Hospital, Chattanooga. They survived 7, 15, and 18½ weeks respectively from the onset of their acute illness. The important features of the clinical course of each are presented in the following summary.

Case 1 (7 Weeks' Survival)

The illness of this white girl, 3 years of age, began with fever, vomiting, and pain in the stomach. This was soon followed by tenderness and rigidity of the neck, increasing weakness of the legs and rapid respiration. She was brought to the hospital on the third day. Examination on admission showed complete flaccid paralysis of both legs, marked weakness of the arms, more on the left, and apparently complete paralysis of the right chest and diaphragm. Function of the cranial nerves appeared normal. She was immediately placed in a Drinker respirator where she remained almost constantly for 3 weeks. After her acute illness subsided, she was able to remain outside the respirator for increasing intervals up to 1 hour. Fluoroscopy during this time revealed paralysis and paradoxical movement of the right diaphragm. While convalescing in the hospital she contracted a mild respiratory infection and responded poorly to treatment. Administration of oxygen and attempted aspiration failed to relieve her respiratory distress and she died 50 days after she was stricken with acute poliomyelitis.

Case 2 (15 Weeks' Survival)

This white girl, 4 years old, entered the hospital acutely ill, extensively paralyzed, and breathing and swallowing with difficulty. Examination on admission showed that only a few weak movements could be made by the arms and legs. The intercostal muscles seemed completely paralyzed and respiration was effected by means of accessory muscles and the diaphragm. She remained in the respirator constantly for 3 weeks during which the acute illness subsided. Following this she gradually became able to leave the respirator, and after 5 weeks was discharged to a convalescent home for physiotherapy and rehabilitation. There she remained 10 weeks

till her final illness. During this time there was little improvement in muscle function. The intercostal muscles were nearly totally paralyzed and breathing was entirely diaphragmatic. Muscles of the extremities were flaccid and atrophic and still showed some tenderness. In the arms, flexion of the fingers and flexion and extension of the wrists were the only active movements possible. Motion in the legs consisted of abduction of the left thigh, and very weak movements of most of the muscles of the right leg. The Babinski sign could be elicited on both sides. As in case 1, this patient contracted a cold which she tolerated poorly because of her impaired respiratory musculature. As her condition became worse, she was placed briefly in a respirator, but she was not helped by it as she seemed unable to accommodate her breathing to the rhythm of the machine. Bronchopneumonia developed, and in spite of the administration of oxygen, sulfathiazole, and a blood transfusion she died on the sixth day of her illness, 106 days after being stricken by poliomyelitis.

Case 3 (18½ Weeks' Survival)

This white boy, 14 years old, was admitted to the hospital on the third day of his illness. At that time he presented incomplete paralysis of both upper and lower extremities. Because of respiratory distress he was placed in a respirator. His acute illness subsided, but he never regained sufficient function of his respiratory musculature to remain for long outside the respirator. After 4 months, however, he had improved sufficiently to be sent home for the Christmas holidays in a respirator. While at home he suddenly had six generalized convulsions within a few hours. He was immediately returned to the hospital, where he died within 24 hours after admission and 130 days after he was first stricken with poliomyelitis.

MATERIAL AND METHODS

Complete autopsies were performed on cases 1 and 2. The brain, the entire spinal cord with most of the thoracic and lumbar root ganglia, the sympathetic chains and the gasserian and celiac ganglia were removed from both and fixed in formaldehyde. From case 3 only the brain and the viscera were obtained for study. Blocks from all three specimens were cut according to a uniform scheme being employed at the National Institute of Health for the investigation of acute poliomyelitis. This, in brief, consisted of cutting large blocks from transverse slices through 30 planes. By this means some 95 blocks were prepared representing fairly completely most of the important structures from the frontal cortex to the caudal end of the medulla. Ten transverse sections were made at representative levels from each spinal cord. The sensory, gasserian, and sympathetic ganglia were cut to obtain the largest surface. All blocks were hardened for 4 days in 2½ per cent aqueous potassium bichromate, embedded in paraffin and sections cut at 8μ. For the general survey a complete set of sections was stained with eosin and methylene blue and thoroughly examined with the aid of a mechanical stage. Glial scarring in selected sections was stained by a modification² of Mallory's phosphotungstic acid hematoxylin method, suitable for tissues fixed in formaldehyde. Alterations in myelin sheaths were demonstrated in celloidin sections of selected

levels of the spinal cord stained by the Weigert-Pal technic. Frozen sections of suitable regions were stained for fat with Sudan IV, and for neuroglia and neurofibrils by various silver impregnation methods.

GROSS PATHOLOGY

Gross examination of the fixed brain specimens during preparation of blocks for section revealed nothing more than some questionable paling of the substantia nigra. Cell loss, chiefly in the medulla, was too subtle to be grossly apparent and alterations in consistence due to gliosis were not perceptible in the hardened tissues. In the anterior horns of the cord, however, there were asymmetric brownish stains extending through a variable number of segments. Such discoloration was probably caused by congested and perhaps numerically increased vessels shining through tissue made more translucent by the loss of ground substance and finer myelinated fibers. At this early stage of repair there was very little gross shrinkage of the anterior horns. Instead, in the most severely damaged lumbar segments, the anterior horns had a loose and very delicate spongy texture, seeming to be almost cystic in some sections.

MICROSCOPIC PATHOLOGY

Cerebral Cortex. Lesions attributable to poliomyelitis were found only in the cortex of the paracentral lobule. In all cases there were one or two small focal accumulations of hypertrophied astrocytes and microglia located principally in the Betz cell layer (Fig. 1). Such scars were chiefly cellular, and only a few neuroglial fibrils were produced. In all cases there seemed to be some diminution of the total number of Betz cells, but the degree of loss was difficult to estimate. Small collars of lymphoid cells were also present about occasional vessels in the cortex of the paracentral regions. Throughout the subcortical white matter, but especially in that beneath the precentral gyri, there were occasional perivascular accumulations of macrophages filled with fat globules or masses of greenish brown, iron-containing pigment.

In case 3 such lesions were overshadowed by the recent thrombosis of a number of small vessels in the cortex at the dorsal end of the central gyri, and the consequent recent infarction of patches of the gray matter. Microscopically this vascular occlusion appeared to have been of very short duration, and quite likely it provided the irritative focus that touched off the patient's generalized convulsions. No anatomic cause for the thrombosis was demonstrable in the sections examined. There was also recent necrosis, without reaction, of Sommers' sector over a considerable length of the left hippocampus. Such a

lesion is occasionally found after convulsive attacks, and now is commonly regarded as a result rather than the cause of the seizures.

No lesions attributable to poliomyelitis were observed in the olfactory bulbs, tracts and trigones or in the hippocampi of any of the three specimens.

Basal Ganglia and Thalami. Even during the height of the acute disease process it is very rare to find more than scanty round cell infiltration about an occasional vessel in the caudate and amygdaloid nuclei, putamen, and claustrum. In the convalescent stage only a few lymphocytes and monocytes, some containing pigment, are to be seen about one or two large veins traversing these nuclei.

The more susceptible globus pallidus and thalamus which regularly show considerable damage in the acute stage, correspondingly present some residual inflammatory changes during convalescence. Their number and character seem to be determined at least partly by the time elapsed since the acute illness. In case 1 after 7 weeks, mild perivascular infiltration and a few loose patches of microglial proliferation were still to be seen in the globus pallidus and thalamus, chiefly along the lateral and ventral margins. In the older cases 2 and 3 cellular reaction in the parenchyma had subsided, and only occasional small perivascular collars of lymphoid cells remained. In all three specimens there were scanty infiltrations of lymphoid cells about some vessels traversing the hypothalamus and anterior perforated substance.

Midbrain. Marked residual inflammatory changes were still present in the midbrain of the first patient 7 weeks after the acute illness. The greatest damage was found in the substantia nigra. Here on both sides there were asymmetric patches in which the ground substance was loose and spongy. In the largest of these, nerve cells had nearly all disappeared, and there was an abscess-like accumulation of foam cells. Nearby vessels were surrounded by broad collars (Fig. 2), while vessels more distant and those traversing the peduncles were occasionally surrounded by mantles of lymphoid cells. In the center of one such foam cell "abscess" was a large multinucleated giant cell of the foreign body type, probably a response to the disintegration products of lipid material. Rarely at the edges of these foci there were coagulated and granular bodies of nerve cells surrounded by phagocytic microglia. Scattered outside the margin were a moderate number of hypertrophied astrocytes. In addition to such areas of destruction there were a few small, focal microglioses in the substantia nigra.

Other structures at the same level presented less severe lesions. In the red nuclei there were a few prominent perivascular collars of lymphoid cells, and occasional small microglial foci and hypertrophied

astrocytes. A very small group of mixed microglia and astrocytes was still evident in the middle and deep layers of anterior colliculi. Two small microglial foci, one surrounding an apparently intact nerve cell, were present in the left oculomotor nucleus.

In cases 2 and 3 of about 4 months' duration the residual inflammatory reaction was much less marked, though of course there is no certainty that damage during the acute illness was equally severe. Both showed occasional collars of lymphoid cells about vessels in the peduncles, substantia nigra and red nuclei. A few small patches of microglial proliferation were present, chiefly near the margin of the red nuclei, and rarely in the substantia nigra and anterior colliculi.

At the level of the posterior colliculi all three cases were practically identical in presenting only mild inflammatory reactions and neuroglial scarring. The chief damage appeared in the lateral tegmental nuclei and caudal end of the substantia nigra. There had been some loss of nerve cells from these structures, and the ground substance contained a moderate number of microglia and scattered hypertrophied fibrous astrocytes. Mild round cell infiltration was present about some of the vessels in these nuclei and in the median raphe. In case 3 only, there were single, small microglial stars in the periaqueductal gray matter and in the left posterior colliculus.

Pons. Three complete transverse sections were made of the pons, one through the anterior medullary velum, one at the level of the trigeminal roots, and one through the abducens nuclei. In all three cases a considerable amount of residual inflammatory reaction persisted in the tegmentum, and as expected the lesions were most marked and acute in character in the case of shortest duration.

At the level of the anterior medullary velum, sections from case 1 showed moderate loss of cells bilaterally in the locus coeruleus, and in their place a considerable proliferation of microglia and foam cells. A similar though less abundant microgliosis was present in the nearby lateral tegmental nuclei. Scattered hypertrophied astrocytes were seen in these lesions, chiefly in the periphery, and a loose irregular patch of microglia and astrocytes appeared in the ventricular floor on the left. Small collars of lymphocytes mixed with some foam cells surrounded a number of vessels in the tegmentum. In sections at this level from the two older cases most of the cellular reaction had subsided, leaving behind scattered hypertrophied astrocytes in the reticular substance, a few small microglial stars, and scanty round-cell infiltration about the regional vessels.

In sections through the trigeminal roots the most obvious lesions were found in the motor nuclei of the V nerve, and unexpectedly they

seemed to be most active in case 3 which had survived $4\frac{1}{2}$ months. In this specimen the lateral half of the right motor V nucleus presented considerable focal and diffuse microgliosis. Several large nerve cells were coagulated and being attacked and removed by phagocytic monocytes (Fig. 3). A few nearby vessels were surrounded by broad collars of foamy and lightly pigmented cells. Among nerve cells at the margin of this lesion were scattered hypertrophied astrocytes (Fig. 4). Some nerve cells had been lost from the motor V nuclei of case 2 and their places were occupied by microglia and a few large fibrous astrocytes. The motor V nuclei of case 1 were apparently intact.

In all three specimens there were scattered, rather prominent perivascular collars in the ventricular floor and reticular substance. Some of the large nerve cells had disappeared, and there was a mild increase of microglia and scattered hypertrophied fibrous astrocytes throughout the reticular substance. No necrotic cells remained but in case 1 a few apparently intact large nerve cells were surrounded by small groups of microglia. No lesions were found in the sensory V nuclei of any of the specimens.

Sections of the posterior part of the pons through the level of the abducens nuclei presented in all three specimens about the maximum degree of destruction of the nervous parenchyma observed throughout the entire brain stem. The most severe damage regularly appeared in the lateral vestibular nuclei of Deiters. The loss of nerve cells was greatest—about 75 per cent bilaterally—in case 1, somewhat less and chiefly on the right side in case 2, and still less in case 3. Corresponding to the cell loss, the nuclei appeared as a somewhat shrunken spongy network of coarse glial fibers and hypertrophied astrocytes (Fig. 5), in the meshes of which were a considerable number of microglia and some foam cells. In case 1 at 7 weeks a few spherical, coagulated masses, apparently dead cell bodies, were embedded in the inflammatory tissue; and a single similar dead cell, without apparent reaction, was still present in Deiters' nucleus of case 3, $4\frac{1}{2}$ months after the onset of poliomyelitis (Fig. 6). Some loss of cells and neuroglial reaction extended irregularly into the adjacent portions of the medial and superior vestibular nuclei. Prominent collars of lymphoid cells surrounded a number of vessels traversing these nuclei.

Damage was present but less severe in the other nuclear masses at this level. As in preceding sections there had been some loss of individual large cells in the reticular substance, and in their places were occasional hypertrophied astrocytes or small groups of microglia. In case 1 only, there was a single abscess-like focus of foam cells surrounded by hypertrophied astrocytes in the ventral part of the right

side (Fig. 7). All three cases presented, on one side or the other, loss of a few cells in the facial nucleus, and in their place some proliferation of microglia and astrocytes. In all there were small collars of lymphoid cells about occasional vessels in the tegmentum. No definite parenchymatous lesions were observed in the abducens and descending V nuclei, or in the superior olives and the gray matter of the basal pons in any of the three specimens.

Cerebellum. Three transverse blocks were cut from the cerebellum of each specimen; one through the anterior medullary velum, one through the center, and one through the caudal third of the hemispheres. Residual inflammatory reaction and scar formation in the cerebellum followed closely the usual pattern of the acute disease. The most evident damage was seen in the tectal nuclei. In all three cases there appeared to have been some loss of nerve cells. The ground substance was loose and spongy and contained an increased number of microglia, some rod cells and a few hypertrophied astrocytes. Regional vessels were surrounded by small collars of lymphoid cells. No parenchymatous lesions were present in the dentate nuclei, but there was a scanty round-cell infiltration about occasional vessels in the hilum. Changes in the cerebellar cortex were strictly limited to the molecular layer of the vermis. Such lesions appeared as irregular vertical streaks of astrocytes and a few microglia crossing the molecular layer. The ground substance was partly dissolved, and among the cells were a moderate number of coarse neuroglia fibrils. Often there was a patch of round-cell infiltration in the overlying meninges (Fig. 8). A few single lesions of this type were present in the first two cases, but none was observed in case 3.

Medulla. Transverse sections were made of the medulla through the VIII nerve roots, at the midpoint of the inferior olives, through the point of closure of the fourth ventricle, and through the decussation of the pyramids.

Lesions at the level of the VIII nerve roots were in general a continuation of those already described in the caudal edge of the pons. Included portions of the VII and lateral vestibular nuclei showed some asymmetric loss of cells and corresponding patchy gliosis. Damage in the reticular substance appeared to be maximal at this level. In case 1 part of the ground substance had disappeared in an abscess-like patch of foam cells. No actual solution of ground substance appeared in cases 2 and 3, but the framework was spongy, somewhat collapsed and contained scattered hypertrophied fibrous astrocytes and small groups of microglia. A number of the large nerve cells had disappeared, and in both cases 2 and 3 there were still single coagulated cells undergoing

neuronophagia 4 months after the acute illness (Fig. 9). It is noteworthy that maximum lesions in the reticular substance appeared in this level, comprising the middle reticular nuclei which had connections with the severely damaged vestibular nuclei and with the anterior horns of the cord. Moderate perivascular infiltration appeared in the affected areas. In the medial vestibular and spinal V nuclei, and rarely in the white matter of the restiform bodies, small microglial stars were to be seen. No lesions were present in the cochlear nuclei.

The middle half of the medulla, as represented by sections through the olives and the closure of the ventricle, presented comparatively mild focal lesions distributed rather irregularly in the dorsal part. In all specimens there was scanty perivascular infiltration, a few small groups of microglia, and scattered astrocytes in the reticular substance and ambiguous nuclei. Little if any loss of nerve cells was demonstrable. One or two cuffed vessels and a few small glial stars were present irregularly in the hypoglossal and lateral cuneate nuclei. In cases 2 and 3 small hemorrhages appeared in the dorsal motor vagus nuclei. Apparently these occurred shortly before death, as there was no reaction to the effused blood, and no visible changes in the adjacent tissue. No lesions were found in any case in the nuclei of the tractus solitarius, the spinal V nuclei, and the inferior olives.

At the level of the decussation of the pyramids the distribution of lesions became practically identical with that in the spinal cord. Damage was chiefly in the anterior horns, where nearly three-fourths of the nerve cells had disappeared, though characteristically the loss was not symmetric. The horns had collapsed somewhat, and the ground substance contained a moderate number of mononuclear (microglial) inflammatory cells. Mingled with them but more numerous along the margins of the horns were a number of hypertrophied astrocytes producing a quantity of coarse glial fibrils. Such fibrous gliosis was more marked in the two later cases (Fig. 10). Prominent collars of lymphoid cells mingled with some fat-laden macrophages surrounded a number of vessels in the anterior horns and central gray matter. There was a mild diffuse hyperplasia of astrocytes in the ventral and lateral white matter surrounding the anterior horns. This appearance was most marked in case 2, in which this portion of the white matter had a coarse, loose texture as if there had been some loss of individual myelin sheaths. In case 1 alone, single small foci of microglia and some astrocytes were present in the nuclei gracilis and cuneatus, while in both cases 1 and 2 a few small glial stars were found in the white matter of the posterior columns. No lesions were present in the spinal V nuclei in any of the three specimens.

Spinal Cord. The entire spinal cord was obtained from cases 1 and 2, but only the first cervical segment was available from case 3. Ten blocks, three each from the cervical, thoracic and lumbar regions and one from the conus medullaris, were cut from each specimen. These were supplanted by other representative blocks taken for special staining methods. As might be anticipated from the extent and severity of the paralysis, both spinal cords presented extensive destruction throughout their entire length. The various levels differed so little in the degree of damage that the spinal cord is best described as a unit.

Loss of the large nerve cells of the anterior horns was almost complete in both cases save at the extreme caudal end of the cord. The few surviving nerve cells were nearly all found along the medial and ventral margins of the anterior horns in the region generally considered to innervate the trunk musculature. In case 1 a few coagulated nerve cell bodies were still to be seen in the lumbar enlargement after 7 weeks (Fig. 11), but in case 2 at 15 weeks the removal of dead cells was practically complete and only rare shrunken masses of uncertain nature remained in the lower lumbar cord.

Damage and loss of nerve cells in the lateral horns appeared to have been initially greater in case 1. In it almost all cells had disappeared from one or the other side in some midthoracic segments. For the most part, however, both cases showed a partial loss of nerve cells in a patchy and asymmetric pattern. Often the disappearance of a few nerve cells was most clearly indicated by the presence of several hypertrophied fibrous astrocytes which filled the gap in the tissue (Fig. 12).

Lesions in Clarke's column appeared only in case 1. Because of the normal irregularity of this structure, the degree of cell loss was difficult to estimate. However, throughout its length there was evidently some patchy disappearance of nerve cells, and in their places were small groups of hypertrophied fibrous astrocytes, as were also seen in the lateral columns. In the transitional zone between thoracic and lumbar regions there was nearly complete destruction of Clarke's columns on both sides. Practically all nerve cells had been removed, and the columns appeared as loose reticular patches of glial fibers containing a number of mononuclear and foam cells as well as large fibrous astrocytes (Fig. 13).

Involvement of the posterior horns evidently resulted secondarily by spreading of the inflammatory process from the anterior horns. Gliosis was most pronounced along the border of the anterior horns in the cervical and lumbar enlargements, and diminished toward the posterior roots. Save for an occasional hypertrophied astrocyte along the root filaments, no definite lesions appeared in the substantia gelatinosa.

Changes in the interstitial tissue of the spinal gray matter were determined by the degree of neuronal damage and by the length of time elapsed since the acute illness. Where only a few cells were destroyed, as in the lateral horns, the ground substance partly collapsed, and nearby astrocytes enlarged and produced a small tangle of coarse glial fibrils. In areas of massive inflammation and destruction of many neurons in the anterior horns, most of the neuropil and fine medullated fibers had disappeared, and their place was taken by a meshwork of glial fibrils. In case 1 at 7 weeks this network was delicate and silky and showed little contraction. The astrocytes laying down the fibrils were bulky with abundant cytoplasm, and were numerous around the margin (Fig. 14). Lying among the glial fibrils were still a moderate number of mononuclear and foam cells containing lipoid debris. Capillary vessels were prominent, though their increase may have been only apparent; and many were surrounded by broad collars of lymphoid and foam cells. In case 2 at 15 weeks the feltwork of glial scar had become more dense, but shrinkage of the anterior horns was only moderate. The glial fibrils were considerably more coarse, and the astrocytes had lost much of their bulky cytoplasm and appeared as dark-stained angular cells (Fig. 15). Scattered mononuclear inflammatory cells still remained. Removal of debris was nearly complete, and only in the cervical and lumbar enlargements where considerable tissue was destroyed were a few lipoid-filled foam cells to be seen. Vessels were less prominent, and perivascular infiltration scanty save for thick collars about a few single vessels.

Damage in the white matter of the spinal cord, as shown in myelin sheath preparations (Fig. 16), consisted of a partial but definite demyelination due to a loss of individual fibers rather than interruption of an entire tract. As expected, it appeared more clearly in the older case 2. In the ventral and lateral columns there was some loss of fibers in all regions save the pyramidal tracts, but the paling was most clearly seen in the superficially placed spinocerebellar tracts. In the dorsal columns the demyelination was more sharply limited to the fasciculus interfascicularis or comma tract of Schultze. Probably much of the fiber loss in the ventral ground bundles was the result of destruction of cells of origin in the anterior horns. However, the degeneration of the spinocerebellar and comma tracts cannot be explained satisfactorily on the basis of their presently accepted connections. Scarcely any cell loss was perceptible in the dorsal root ganglia and posterior horns, and though a number of cells had been destroyed in Clarke's column in case 1, demyelination in the dorsal spinocerebellar tract seemed slightly less than in case 2 in which Clarke's column was almost intact.

Routinely stained sections showed a mild diffuse hyperplasia of astrocytes in the demyelinated areas, and there was probably a slight increase in size and number of glial fibrils.

Nerve Roots. The anterior nerve roots showed in striking manner the result of extensive destruction of motor nerve cells. In myelin sheath preparations (Fig. 17), more than 80 per cent of the myelinated fibers had disappeared. The loss of the coarse motor nerve fibers was nearly complete. Remaining fibers were, for the greater part, of small diameter with thin myelin sheaths, and most were probably the axons of efferent sympathetic neurons. This sparing of the sympathetic components of the anterior roots was especially striking in the thoracic segments where (Fig. 18), as was noted above, there was comparatively little diminution of nerve cells in the lateral horns.

Differences in the stage of nerve root degeneration due to the duration of the process may be observed in Weigert preparations, but are more clearly shown in ordinary stained sections. In the nerve roots from case 1 at 7 weeks there were still a number of phagocytes containing globules of lipoid material, some of which stained as myelin. In case 2 after 15 weeks no stainable globules remained, and the roots contained only the scattered surviving myelinated nerve fibers.

In routine sections of case 1 the anterior roots appeared moderately reduced in size and abnormally cellular. Under higher magnification (Fig. 19), the increased cellularity was seen to be due partly to proliferation of Schwann cells and endoneurial fibroblasts, but chiefly to accumulation of numerous foamy macrophages. These were arranged in bead-like rows, filling the endoneurial nerve tubes once occupied by large myelinated fibers. The phase of active proliferation of these cells was evidently passed since no mitotic figures were present.

The anterior roots of case 2 showed a more advanced stage of atrophy (Fig. 20). Their total size was greatly reduced. The endoneurial connective tissue seemed coarser and more abundant, though much of the apparent increase may have been due to collapse and shrinkage. Nerve tubes which had lost their fibers generally contained scattered Schwann cells. Some of the larger ones in cross section appeared quite empty. Only rarely were one or more foamy macrophages still to be seen. In both cases a scanty infiltration of lymphoid cells was found about a few of the small endoneurial blood vessels.

No significant lesions were found in the posterior nerve roots of either case.

Cerebrospinal Ganglia. Both gasserian ganglia and four representative pairs of dorsal root ganglia were examined from cases 1 and 2. In case 1 occasional small foci of lymphoid cells were still present after 7

weeks, in the stroma of both gasserian and root ganglia. Rarely in the lumbar ganglia a single nerve cell had disappeared, and its capsule was filled with proliferated satellite cells (Fig. 21). In case 2 after 15 weeks no lesions were found in the gasserian ganglia, and in the root ganglia there were only very rare small foci of lymphocytes.

Sympathetic Ganglia. Both thoracolumbar sympathetic chains and the celiac ganglia from both cases were examined. In each case the celiac ganglia contained a very few small foci of lymphoid cells, and in case 2 there were a few eosinophilic leukocytes among the bundles of nerve fibers. No lesions were found in the thoracolumbar sympathetic chains in either case.

Meninges and Choroid Plexus. Inflammatory reaction in the meninges was limited to a few scanty patches of lymphoid and mononuclear cells, not apparently related in any close fashion to lesions in the nervous parenchyma. In case 3 alone, the tips of the arachnoidal villi along the longitudinal fissure contained a moderate number of polymorphonuclear leukocytes. This unusual reaction was most probably a response to blood and irritative products of tissue disintegration seeping out from the patches of recent infarction in the paracentral lobules. No significant lesions were found in the choroid plexus of any of the specimens examined.

ATTEMPT AT VIRUS ISOLATION

A small piece of the cervical cord of case 3 was preserved in glycerine-saline solution to test for the presence of virus. Unfortunately the specimen was contaminated with bacteria not killed by the glycerine, and two monkeys inoculated intracerebrally died promptly of acute brain abscess, too early to show any possible evidence of poliomyelitis. Accordingly, the question of persistence of virus in the nervous system during convalescence remains unanswered. Since inflammatory reaction and a few necrotic cells are still found after 18½ weeks, it would appear desirable to do animal inoculations for virus whenever the rare opportunity is afforded of an autopsy on a patient in the convalescent stage of poliomyelitis.

SUMMARY

The pathology of the residual lesions in three cases of poliomyelitis which had survived 7, 15 and 18½ weeks from onset of illness has been described and illustrated.

Lesions in the cerebral cortex consisting of perivascular collars of lymphoid cells and interstitial foci of microglia and astrocytes are confined to the paracentral lobules. Some diminution of Betz cells also

appears probable. In case 3, only, there was thrombosis of small vessels and patches of recent cortical infarction in the central convolutions at the vertex.

Only minimal lesions are found in the basal ganglia and thalami. In the earliest case, scanty perivascular infiltration and a few small microglial foci appear in the globus pallidus and ventral and lateral part of the thalamus. In the two later cases slight perivascular infiltration, only, is seen in these structures.

In the midbrain the substantia nigra presents the most severe damage. A few necrotic cells are still undergoing phagocytosis in case 1 at 7 weeks. Irregular perivascular infiltration, and interstitial foci of mixed astrocytes and microglia, appear in the lateral tegmental and red nuclei, generally in amount decreasing with the time elapsed since the initial illness.

Lesions in the pons are confined to the tegmentum. Loss of nerve cells is marked in Deiters' nuclei, and more patchy and asymmetric in the motor V and VII nuclei. Single necrotic cells are still present 4 months after the acute illness. Perivascular infiltration diminishes and the density of fibrous gliosis increases with the duration of convalescence. Part of the large nerve cells of the reticular substance have disappeared, leaving behind scattered large fibrous astrocytes. In case 1 part of the ground substance, as well as cells of the reticular substance, has been destroyed, leaving an abscess-like accumulation of foam cells.

In the cerebellum, lesions are found only in the tectal nuclei and in the cortex of the vermis. Some cells of the tectal nuclei have disappeared, and there is a patchy hyperplasia of astrocytes. In the vermis a few vertical streaks of astrocytes and microglia extend across the molecular layer of the cortex.

The most marked changes in the medulla consist of cell loss and scarring in the reticular substance similar to that found in the pons. Rare dead cells are still present in cases 2 and 3. Asymmetric glial patches are found in the ambiguus and hypoglossal nuclei. At the pyramidal decussation the lesions become practically the same as in the spinal cord. Only a few small glial stars appear in the nuclei gracilis and cuneatus and in their tracts.

The spinal cord presents an almost complete loss of nerve cells throughout the entire length of the anterior gray substance. In contrast the lateral horns are comparatively spared, lesions in Clarke's column are patchy and asymmetric, and no definite changes appear in the posterior horns. Replacement gliosis in the anterior horns is at first abundant but delicate, with bulky astrocytes. Later the cells

shrink and the fibrils become coarser. Numerous mononuclear inflammatory cells and rare necrotic nerve cells are still present in case 1, but have largely disappeared from the later cases.

In the white matter of the spinal cord there is a mild diffuse demyelination of most of the ventral and lateral columns with the exception of the pyramidal tracts. In the posterior columns partial demyelination is confined to the region of the comma tracts of Schultze. Much of this tract degeneration is due to factors other than loss of the known cells of origin.

The anterior nerve roots show severe degeneration consequent to the extensive loss of anterior horn cells. Almost all of the coarse motor fibers have disappeared. In contrast the fine myelinated efferent sympathetic fibers are mostly spared. At 7 weeks the nerve tubes contain many fat-laden phagocytes, but by 15 weeks nearly all phagocytes have disappeared, the roots are shrunken and the endoneurial connective tissue is somewhat increased. No changes are recognizable in the posterior roots.

In the gasserian, dorsal root and sympathetic ganglia there are a very few small foci of lymphoid cells. In the root ganglia only rare cells have disappeared, leaving behind capsules filled with mononuclear cells.

The meninges contain only a few scanty foci of lymphoid cells, and no lesions were found in the choroid plexus.

An attempt in case 3 to detect virus in a small portion of glycerinated spinal cord was frustrated by bacterial contamination of the inoculum.

Pathologic specimens and clinical data of cases 1 and 2 were furnished by Dr. G. John Buddingh, Department of Pathology, Vanderbilt University. Corresponding material of case 3 was contributed by Dr. Forrest G. Bratley, pathologist of the Erlanger Hospital, Chattanooga, Tennessee. The author is pleased to acknowledge his indebtedness to these pathologists whose generous donation of their specimens made possible this report.

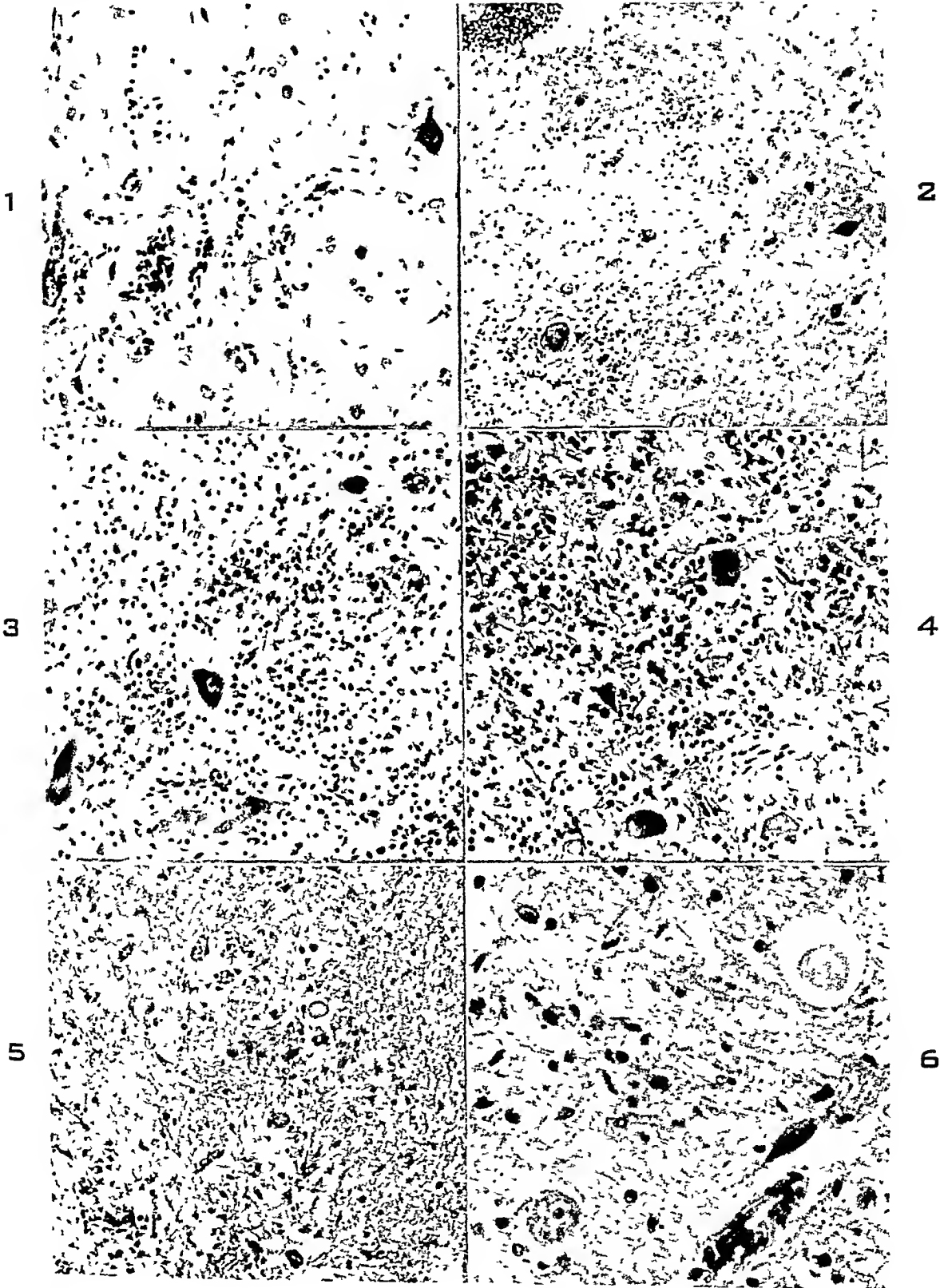
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1. Warburg, B. Experimental poliomyelitis; histology of the persistent lesions of the central nervous system. *Arch. Neurol. & Psychiat.*, 1931, 25, 1191-1232.
2. Peers, J. H. A modification of Mallory's phosphotungstic acid-hematoxylin stain for formaldehyde-fixed tissues. *Arch. Path.*, 1941, 32, 446-449.

DESCRIPTION OF PLATES

PLATE 78

- FIG. 1. Glial nodule in the motor cortex of case 1 (7 weeks). Eosin and methylene blue stain. $\times 155$.
- FIG. 2. Substantia nigra, case 1 (7 weeks), showing cell loss and foreign body giant cell. Eosin and methylene blue stain. $\times 105$.
- FIG. 3. Motor V nucleus of case 3 ($18\frac{1}{2}$ weeks), showing diffuse microglial infiltration and phagocytosis of a dead cell. Eosin and methylene blue stain. $\times 143$.
- FIG. 4. Same as in Figure 3 showing a few hypertrophied astrocytes and irregular feltwork of neuroglial fibrils. Phosphotungstic acid hematoxylin stain. $\times 183$.
- FIG. 5. Deiters' nucleus, case 2 (15 weeks), showing marked loss of nerve cells and collapsed and spongy ground substance with hypertrophy of astrocytes at the periphery. Phosphotungstic acid hematoxylin stain. $\times 143$.
- FIG. 6. Deiters' nucleus, case 3 ($18\frac{1}{2}$ weeks). A necrotic nerve cell without cellular reaction is seen in the upper right. Hypertrophy of astrocytes. Eosin and methylene blue stain. $\times 285$.



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PLATE 79

FIG. 7. Reticular substance of medulla, case 1 (7 weeks), showing diffuse microgliosis, loss of nerve cells and small foam cell "abscess." Eosin and methylene blue stain. $\times 80$.

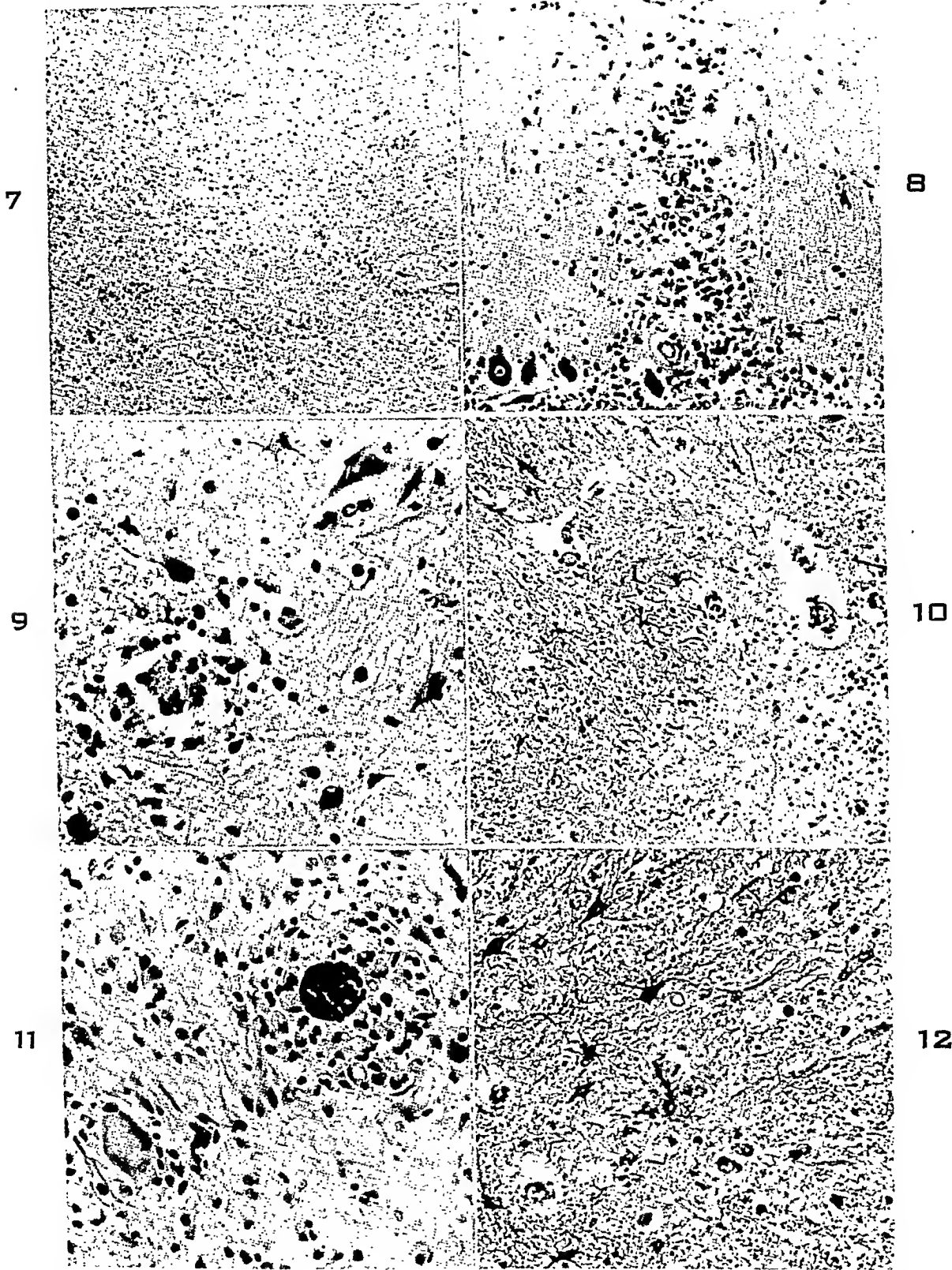
FIG. 8. Cerebellar cortex, case 3 (18½ weeks), showing vertical streak of microglia and astrocytes crossing the molecular layer. Eosin and methylene blue stain. $\times 155$.

FIG. 9. Reticular substance of medulla, case 3 (18½ weeks), showing phagocytosis of a dead nerve cell and hypertrophy of surrounding astrocytes. Eosin and methylene blue stain. $\times 310$.

FIG. 10. Upper cervical cord, case 3 (18½ weeks), showing hypertrophied fibrous astrocytes at margin of anterior horns. Phosphotungstic acid hematoxylin stain. $\times 143$.

FIG. 11. Lumbar cord, case 1 (7 weeks), showing dead motor nerve cell, broad perivascular collar of foam cells and early gliosis in ground substance. Eosin and methylene blue stain. $\times 285$.

FIG. 12. Thoracic cord, case 1 (7 weeks), showing hypertrophied astrocytes in the lateral horn. Phosphotungstic acid hematoxylin stain. $\times 285$.



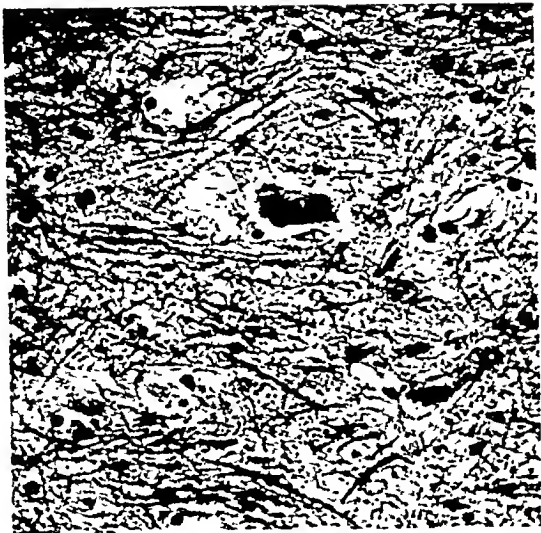
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PLATE 80

- FIG. 13. Thoracic cord, Clarke's column, case 1 (7 weeks), showing microgliosis and hypertrophy of astrocytes. There is a dead cell body in the upper left portion of the figure. Phosphotungstic acid hematoxylin stain. $\times 285$.
- FIG. 14. Lumbar cord, anterior horn, case 1 (7 weeks), showing monocytes and foam cells in ground substance and about vessel; hypertrophy of astrocytes with sparse and irregular formation of neuroglial fibrils. Phosphotungstic acid hematoxylin stain. $\times 285$.
- FIG. 15. Lumbar cord, anterior horn, case 2 (15 weeks), showing abundant meshwork of coarse neuroglial fibrils and less bulky astrocytes. There are few remaining inflammatory cells. Phosphotungstic acid hematoxylin stain. $\times 285$.
- FIG. 16. Cross section of thoracic cord, case 2 (15 weeks), showing partial demyelination of comma tracts, and of most of the medial and ventrolateral white columns. Weigert-Pal stain. $\times 10$.

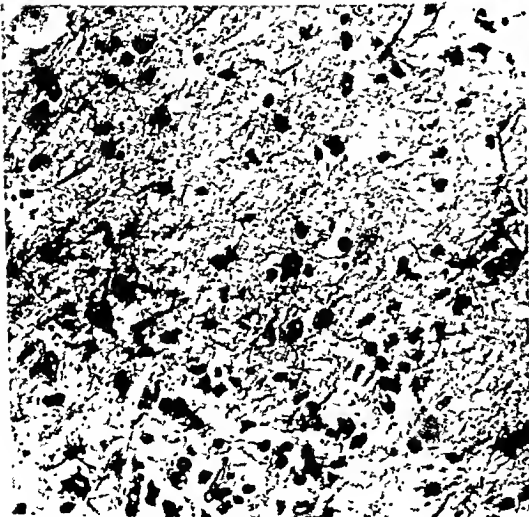
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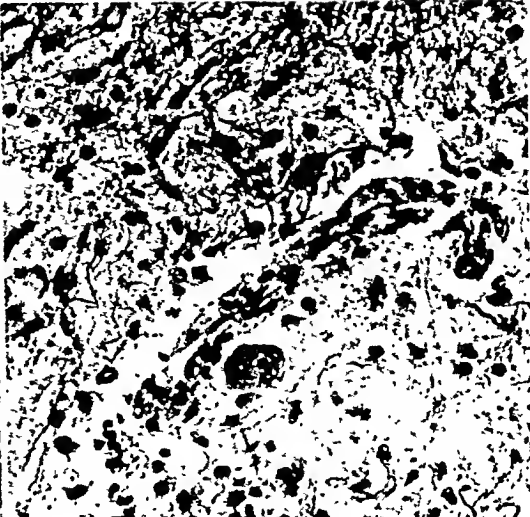
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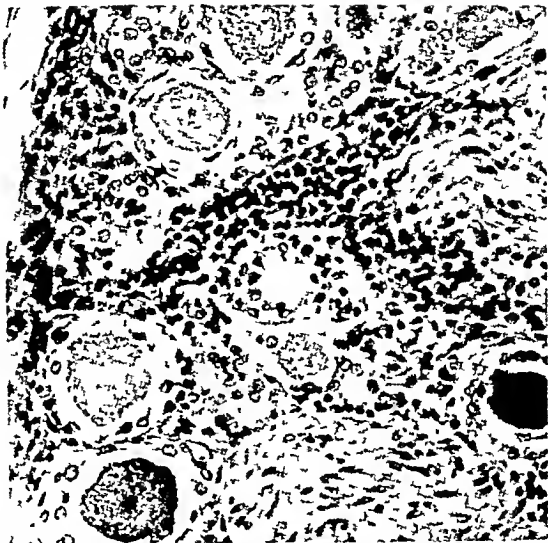
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Convalescent Poliomyelitis in Man

PLATE 81

- FIG 17. Anterior nerve root, lumbar region, case 2 (15 weeks), with loss of nearly all large myelinated nerve fibers. Weigert-Pal stain. $\times 75$.
- FIG. 18. Anterior nerve root, thoracic region, case 1 (7 weeks). There is nearly complete loss of large myelinated fibers. Fine medullated sympathetic fibers are practically intact. Weigert-Pal stain. $\times 75$.
- FIG. 19. Anterior nerve root, lumbar region, case 1 (7 weeks), showing fat-laden phagocytes occupying empty nerve tubes. Eosin and methylene blue stain. $\times 270$.
- FIG. 20. Anterior nerve root, lumbar region, case 2 (15 weeks), showing marked shrinking and some apparent increase of endoneurial connective tissue. Few reacting cells remain. Eosin and methylene blue stain. $\times 270$.
- FIG. 21. Dorsal root ganglion, lumbar region, case 1 (7 weeks), showing an interstitial focus of lymphocytic infiltration near the capsule. Eosin and methylene blue stain. $\times 235$.

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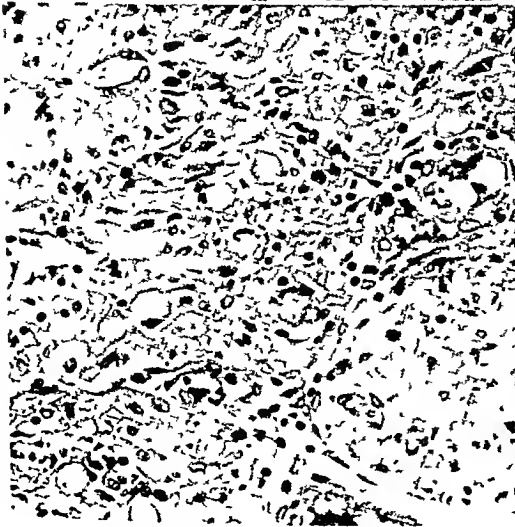
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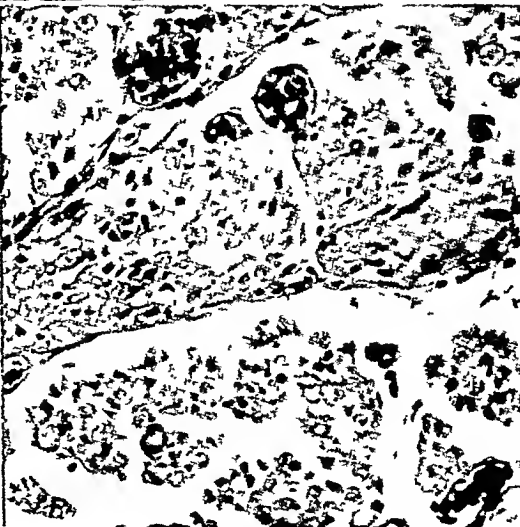
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ATROPHY OF THE BRAIN FOLLOWING PUERPERAL ECLAMPSIA *

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Mental deterioration following puerperal eclampsia is little known. Amand (1790), according to Sioli,¹ described a young woman who "lost her mental faculties to such an extent as to become unable to read and write and even monogram her linen, all things she had been able to do before." In modern literature there is only the clinical contribution by Nevermann,² who reported mild aphasia and moderate mental deterioration 5 years after childbirth complicated by eclampsia. We were unable to trace any patho-anatomic investigations on the subject and wish to present a case of advanced atrophy of the brain caused by this disorder.

REPORT OF CASE

Clinical History. A white married woman, 20 years of age, a primipara, gave birth to a child on March 21, 1930. According to the statement of the attending physician the pregnancy was uneventful until 3 days before delivery, when headaches occurred, the urine was found to contain large quantities of albumin and the blood pressure rose to 200/90 mm. of Hg. However, the delivery was rapid and uneventful, resulting in a living, normal child. One hour later there occurred almost continuous uncontrollable convulsive attacks which persisted for 14 hours. The patient remained comatose for 60 hours, regaining consciousness gradually. Ninety-six hours following delivery she opened her eyes, occasionally moved her extremities and swallowed liquids, but did not respond to questions, did not recognize relatives and did not talk. The pupils were enlarged (examination of the eyegrounds was not possible); the muscles were generally flaccid, but at times those of the neck and of the right arm were rigid and twitched spontaneously or on touch. All reflexes were exaggerated, and no pathologic reflexes were present. The liver was enlarged and was felt 5 cm. below the costal margin. There followed a slow and only partial physical recovery. Within 10 days the blood pressure returned to normal and the excretion of urine increased to 1200 cm. daily. (There is no statement concerning the blood pressure and flow of urine during the first days of the disease.) Ten weeks after delivery the heart rate ranged between 110 and 140; the blood pressure was 124/94; the rate of respiration was 12 per minute. The urine still contained albumin and a large number of white blood cells. The liver was 2.5 cm. below the costal margin.

The patient continued in a state of mental deterioration. She gradually became able to swallow solid food when it was placed in her mouth, and reacted to painful stimuli but made few spontaneous movements. The eyes followed a moving object, the mouth was spontaneously opened and closed, teeth clicked and a few unintelligible sounds were muttered. Most of the time she lay quietly in bed, paying no attention to her surroundings and not responding to stimuli or questions. Later, she was able to sit in a chair. She continued to lead a vegetative existence until her death, 6 years later, on July 3, 1937.

* Received for publication, October 5, 1942.

Gross Examination

Postmortem examination revealed: (1) chronic suppurative pyelonephritis on the right (with streptococci in smears); (2) amyloidosis of kidneys, liver, spleen and myocardium; (3) an old tuberculous lesion in the apex of the left lung.

The brain weighed 579 gm. There was a severe bilateral atrophy of the frontal, temporal and parietal lobes (Fig. 1). The precentral and postcentral gyri and the occipital lobes were only moderately atrophic (Fig. 1). In the left frontal lobe all gyri of the convexity were atrophic, the atrophy being less pronounced at the base and on the median surface. In the right frontal lobe the atrophy was equally severe over the convexity, median surface and the base. In the left temporal lobe all gyri of the convexity and those of the base with the exception of the gyrus hippocampi were atrophic (Fig. 1); the atrophy extended into the parietal region, severely involving the angular and supramarginal gyri and large parts of the superior parietal gyrus (Fig. 1), and continued into adjacent parts of the occipital lobe. In the right temporal lobe all gyri with the exception of the midportion of the superior temporal gyrus and the gyrus hippocampi were destroyed, the atrophy continuing into the left angular and supramarginal gyri. Coronal sections showed a most severe cystic degeneration of the white matter throughout the brain, which in many areas exceeded that of the gray matter, the latter being frequently preserved (Fig. 2). Only at the base of both temporal lobes and in the right frontal lobe was the destruction equally severe in both the gray and white matter, reducing the convolutions to thin-walled cysts.

The caudate body, putamen and pallidum were moderately atrophic and the entire thalamus was greatly atrophic. The hypothalamus and the dorsal part of the pons were of normal size and appearance. The basilar part of the pons and numerous folia of the cerebellum were distinctly reduced in size.

Weigert preparations revealed advanced demyelination in all atrophic areas, especially so in both parietal lobes and at the base of both temporal lobes, while in both superior temporal convolutions, operculum and hippocampal gyri a considerable amount of myelin was still preserved (Fig. 3). There was also considerable reduction of myelin in the corpus callosum, fornix and the mammillary bodies and only mild demyelination in both occipital lobes and in the cerebellum.

Microscopic Examination

Histologic examination of the cortex showed preservation of a considerable part of the parenchyma. Even in the most severely destroyed

areas remnants of the cyto-architecture were visible. Usually the two or three upper layers could still be distinguished, while the lower layers were replaced by a spongy tissue (Fig. 4). In areas less severely affected usually the upper four layers survived, while in the motor cortex, operculum, occipital lobes, and in the islands the entire cyto-architecture could be recognized. In these latter areas there were numerous small perivascular foci of parenchymatous degeneration of the type described by Spielmeyer.³ The neurons throughout the cortex showed various types of degeneration. In the most severely affected areas they were greatly shrunken; in others the so-called ischemic degeneration, frequently associated with incrustations, prevailed. There were changes in the neurons of the corpus caudatum, putamen, and in a milder form in the pallidum, similar to those of the cortex. However, there was no appreciable reduction in the number of nerve cells with the exception of the caudate nucleus. In the thalamus there was an extensive secondary degeneration of the parenchyma which was replaced by glial scars, especially in the anterior and lateral nuclei.

As already mentioned, the degeneration of the white matter was more severe than that of the gray, the severe destruction being present in the marginal strata and less frequently in the deeper parts (Fig. 4). In numerous areas there was a spongy state (Fig. 4), built of a delicate network of glial fibers occasionally interspersed with astrocytes (many of them of the giant variety), capillaries, a few gitter cells, scattered axis cylinders and remnants of myelin sheaths. The hypothalamus and the nuclei of the brain stem were normal, with the exception of the pontile nuclei and inferior olives, which were moderately degenerated. In the cerebellum there were extensive focal areas of degeneration in the cortex of both hemispheres, in the vermis, and in the dentate nuclei, and a diffuse moderately advanced demyelination in both semilunar lobes.

The resorption of the destroyed tissue had been completed everywhere and there were no signs of tissue activity except for small scattered foci of proliferating microglia and a few gitter cells. Lipoids and pigments were not seen. The meninges were greatly thickened and fibrotic over all degenerated areas (Fig. 4). The blood vessels of the entire central nervous system showed no definite alterations with the exception of terminal thrombi in the large veins, but there were no remnants of old organized or recanalized thrombi or of old hemorrhages.

Numerous commissural, projection and association systems showed secondary degeneration. Incompletely demyelinated systems stood out in Weigert sections, while completely degenerated pathways could be

visualized in Nissl preparations because of gliosis. There was advanced gliosis in the cingulum, in the uncinate and in the superior longitudinal fasciculi. The corpus callosum and the anterior commissure were considerably demyelinated. Of the projection systems, the frontal, temporal and parietal thalamic radiations were degenerated as were also the frontopontile and temporo-pontile tracts. The pontocerebellar fibers were distinctly reduced in number. There was only a moderate reduction of myelin in the corticospinal tracts. The auditory and optic radiations were normal.

COMMENT

This case belongs to a variety of puerperal eclampsia which is characterized by a particularly severe destruction of one organ such as liver (Konstantinowitsch,⁴ Ceelen⁵), kidneys (Klotz,⁶ Bradford and Lawrence,⁷ Griffith and Herringham,⁸ Lloyd,⁹ Jardine and Teacher,¹⁰ Schüppel¹¹), heart (Ottow¹²), or brain, as in this case. If we assume that the normal brain weight in our patient (who was 167 cm. tall) was approximately 1300 gm., we must conclude that at the time of death 60 per cent of the brain substance was resorbed.

The pathologic findings in the brain can be divided into primary and secondary. To the first group belong all changes directly caused by abnormalities of the metabolism of unknown nature during the acute phase of the disease; to the second such changes as appeared later secondary to extensive degeneration of the white and gray matter. To the former belong acute destruction of marginal layers of the white matter of the hemispheres, of the lower layers of the cortex, and of the gray matter of the cerebellum, and to the latter the degenerative changes of the commissural, association and projection systems.

For the understanding of the patho-anatomic changes it is important to bear in mind that the most advanced primary degeneration occurred in the marginal zones of the gray and white matter in which the destruction was so complete that remnants of the glial tissue which survived were able to build only a poorly organized spongy scar. This almost universal development of the marginal degeneration virtually severed the cortex of the hemispheres from the white matter, producing a picture closely resembling that described by Nissl¹³ following experimental decerebration in rabbits. This dissociation of the cortex from the white matter occurred in extensive areas of the frontal, temporal and parietal lobes, *i.e.*, those parts of the brain which are mainly concerned with higher mental activity, accounting for the sudden and profound mental deterioration.

Interpretation of the pathophysiologic mechanism responsible for

the atrophy is difficult since at the time of death the destroyed tissue was resorbed, no longer permitting a comparison with histologic changes in acute stages of this disease. According to our present state of knowledge, the latter is characterized by (1) diffuse toxic degeneration of the parenchymatous cells with disintegration of the cyto-architecture, (2) small areas of necrosis in the gray and white matter, (3) hemorrhages, (4) thrombosis, frequently with "hyaline" degeneration of the vessel wall, and (5) edema of the brain.

None of these findings can satisfactorily explain the unusual extent of damage in the present case. There is no evidence that the destruction was due to thrombosis since no remnants of old thrombi were found and the vessels appeared to be normal, nor could hemorrhages taking place during the acute stage be made responsible for the atrophy since no accumulation of blood pigment or other remnants of organized hemorrhages were present.

It seems reasonable to assume that the lesions were the result of a primary necrosis of the tissue, similar to the case of cardiac degeneration of Ottow,¹² and the question arises as to the manner in which it took place. Two possible pathogenetic factors, or a combination of them, must be considered: (1) functional disturbances of circulation, (2) toxic influences.

Functional disturbances of the circulation, such as angiospasm, have been considered an important factor in eclampsia. Bodechtel¹⁴ described degeneration of the calcarine area, which he attributed to such disturbances since the vessels in the destroyed area showed no organic changes. This view was challenged by Heynemann,¹⁵ who pointed out that similar vasospasms are also present in other disorders which have neither etiologically nor pathologically anything in common with eclampsia, such as atherosclerosis of the kidneys, glomerulonephritis, and many others. He concluded that the "vascular factor" is merely a symptom of eclampsia and is pathogenetically of no significance. While Heynemann's conclusions may apply to renal conditions, it is doubtful whether they can apply to the pathology of the brain. Spielmeyer³ showed conclusively that functional changes in circulation may very well cause focal degenerative lesions in the cortex under a variety of conditions, such as physical trauma and hypertension, and also in eclampsia and other intoxications. The morphology of the lesions is always similar and permits no differential diagnosis. While the lesions described by Spielmeyer were limited in size, Malamud and Boyd¹⁶ demonstrated that functional disorders in vessels which are morphologically apparently normal may cause a breakdown of the entire vasomotor system of the cortex resulting in extensive

ischemic necrosis. It must therefore be considered as likely that functional disorders of the circulation may produce extensive cortical damage, but this is not known to have occurred in the white matter, which is so extensively damaged in our case. Functional disturbances of vascular nature unquestionably did occur in the acute stage, since small perivascular foci of degeneration were present in many areas of the cortex, but these foci remained small and can in no way account for the extensive degeneration either of the gray or the white matter nor contribute to the understanding of the pathogenesis. Since the white matter is much less rich in vessels and is therefore much less dependent on oxygen supply, it is difficult to attribute the necrosis of the subcortical tissue to the "vascular factor." Some considerations seem to suggest a different pathophysiologic mechanism: the destruction of the white matter is most severe in the marginal (subcortical) layers, *i.e.*, in those parts in which toxic influences are frequently at work. In this connection we call attention to conditions in which disturbances of metabolism cannot be denied, such as Wilson's disease and many other disorders of the liver in which the degeneration of the marginal layers of both gray and white matter attain extensive proportions. Similarly, in fatal nitrous oxide poisoning the same areas of the brain are destroyed. Therefore, it seems logical to assume that toxic influences are mainly responsible for the destruction of brain tissue in this case.

While the permanent damage was confined to the brain, the pathologic changes of the internal organs are of some importance, as contributing factors. The enlargement of the liver in the early days of the disease suggests acute involvement of this organ, which was later relieved, while the pyelonephritis apparently assumed a chronic course finally resulting in amyloidosis in various organs.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 82

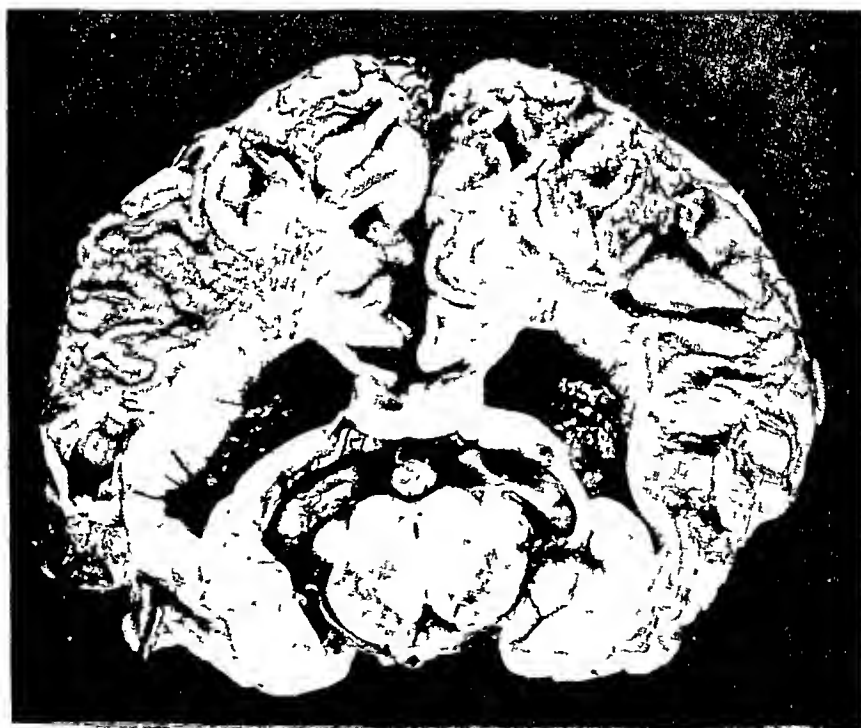
FIG. 1. Severe atrophy of the frontal, temporal and parietal regions.

FIG. 2. Advanced cystic atrophy of the white matter in both hemispheres; cortex severely involved in both temporal lobes.

1



2



Lowenberg and Lossman

Atrophy of the Brain Following Eclampsia

PLATE 83

FIG. 3. Weigert preparation, demonstrating demyelination in both parietal and large parts of the temporal region.

FIG. 4. Nissl preparation, demonstrating spongy state in the marginal stratum; meninges considerably thickened. $\times 20$.

3

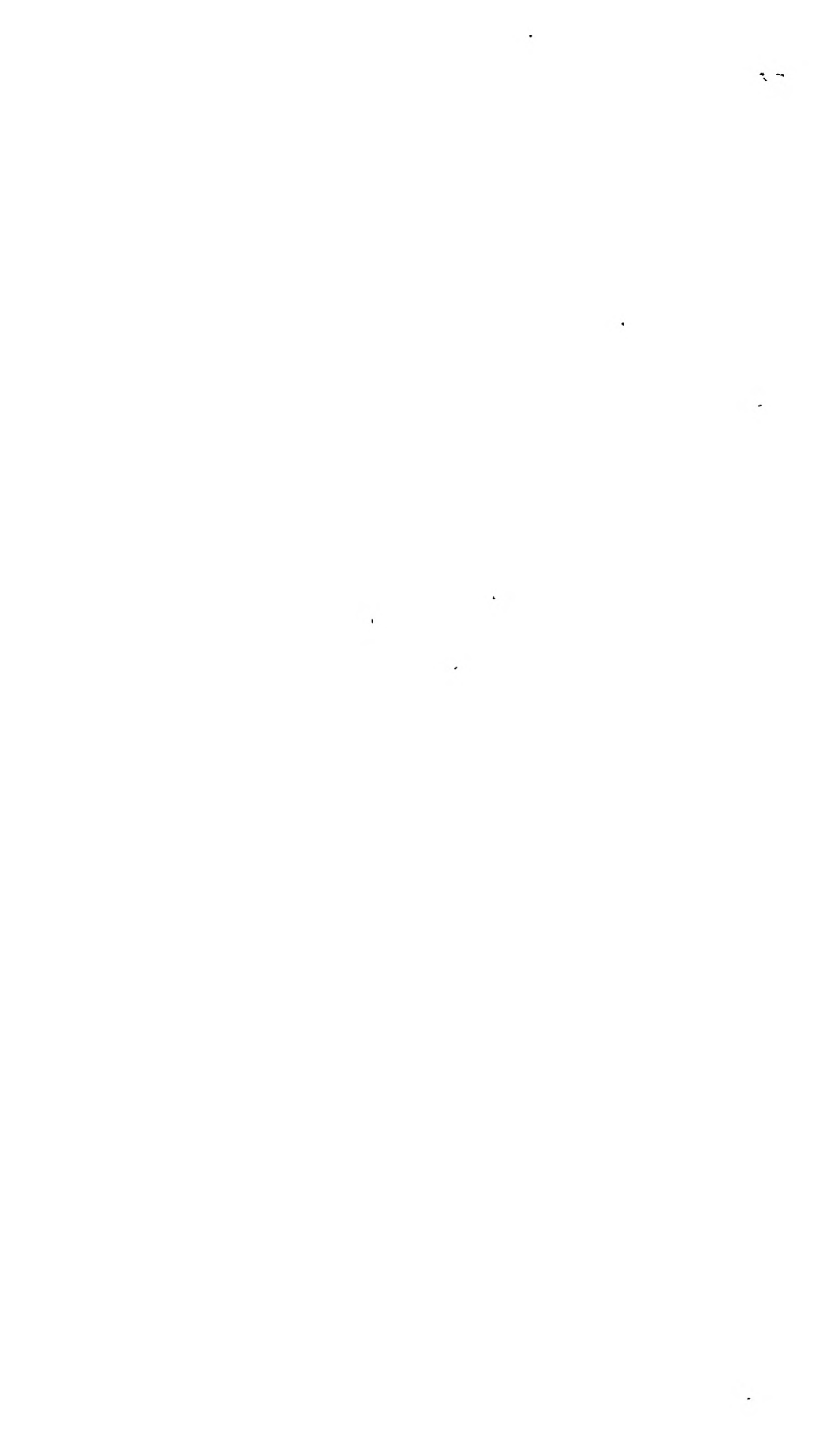


4



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Atrophy of the Brain Following Eclampsia



MEDULLARY INVOLVEMENT IN TETANUS *

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In recent literature, reports of histopathological studies of the alterations that occur within the nervous system in tetanus are lacking. This is unusual when one considers the frequency of this disease and the large number of studies on its pathogenesis and treatment. No doubt one of the chief reasons for this paucity of pathological studies is the general impression that tetanus produces very little anatomical damage to the nervous system. Many investigators have reported such negative observations in spite of accurate studies (Wagner,¹ Leyden,² Tauber,³ Vincenzi,⁴ Nageotte and Ettlinger,⁵ Courmont, Doyen and Paviot⁶ and Sjövall⁷). However, it is difficult to believe that a disease which produces such dramatic neurological symptoms can avoid leaving structural damage in at least a few of the more severe cases. It seems much more likely that this disease is often so fulminating in its course that insufficient time elapses for the actual tissue changes to become microscopically discernible. It was for this reason that detailed pathological studies were undertaken in 12 fatal cases of tetanus, in an attempt to locate and, if possible, correlate any discovered changes of the nervous system with the durations of the illness.⁸ It was apparent from these studies that in all cases of tetanus in which the illness lasts over 3 days, definite changes do occur within the nervous system. Up to the fifth day of the illness these changes are limited to the nerve cells and consist of mild swelling and chromatolysis. If the patient survives beyond the fifth day, there may occur much more extensive lesions consisting of perivascular glial nodules, and perivascular demyelination. At the time of these original studies, my interest was attracted by the tendency of the lesions, in some cases, to be most intense within certain cranial nerve nuclei. Such a selective localization frequently correlated fairly closely with many of the clinical symptoms presented by the patients. In order to study more carefully this distribution of the cellular lesions, serial sections were made of all the cranial nerve nuclei, of the spinal cord at various levels and of various cortical areas in a case of tetanus, showing what appeared to be definite signs of medullary involvement throughout the course of the illness. The findings in this case comprise the present report.

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REPORT OF CASE

W. P. (hospital no. 722000), a farmer, 41 years old, had a wart removed from his left middle finger 2 weeks before admission to the hospital. He continued to work on his farm and felt well until 10 days later when he first noticed some stiffness in his jaw. By the next day he was unable to open his mouth and had some difficulty in swallowing. He became restless and irritable and was taken to the local hospital where he received some tetanus antitoxin. In spite of this therapy, he continued to show a progression of symptoms, developing periodic attacks of generalized tonic spasms of most of his musculature. On the third day of his illness he was transferred to the University Hospital. On admission the patient was conscious and co-operative, but the slightest stimulus precipitated a generalized tonic spasm of most of his musculature. He had a marked trismus. Even at this early period it was noticed that the patient had definite cardiac and respiratory irregularity in spite of the absence of any discernible pathology in these organs. His breathing was particularly involved, at times being extremely slow or very rapid, shallow and labored and sometimes being normal. The pulse also varied from a firm, full regular beat to rapid regular or irregular pulsations which occasionally were difficult to palpate. The patient was treated with tetanus antitoxin intravenously, receiving 80,000 units during the first 48 hours of hospitalization. His muscular spasms were so well controlled by avertin that throughout most of his stay he remained well relaxed. In spite of this medicinal control of the muscular involvement, the cardiac and respiratory irregularities nevertheless continued to become more severe. His respirations became more labored and irregular and the patient expired, apparently from medullary paralysis, after a hospital stay of 3 days and on the sixth day of his illness.

Pathological Studies

There were no gross changes within the nervous system.

Histological Studies

Sections from various parts of the nervous system were prepared by Nissl's, Weil's and the hematoxylin-phloxin B technics.

Cerebral Cortex. The gray matter of the hemispheres revealed surprisingly few changes. Most of the nerve cells were intact (Figs. 1 and 2). An occasional cell showed mild perinuclear chromatolysis. In a few cells, the perinuclear Nissl granules, although present, were very fine while the peripheral granules were large and heavily pigmented. In none of the neurons were changes noted either in the nuclei or in cell processes.

There were no alterations within the glial elements. Many of the small vessels showed a moderate degree of endothelial swelling and some proliferation, but in none was the lumen markedly attenuated or closed.

Cerebellum. Many of the Purkinje cells revealed a mild reduction in the intensity of their staining properties but were otherwise structurally intact. There were no changes within the granular elements or within the white matter.

Midbrain. The large cells of the lateral oculomotor nuclei were intact. Their Nissl granules were large and irregular and stained very deeply (Fig. 3). Dorsal to these cells were the small pyriform cells of the nucleus of Edinger and Westphal. Normally these cells contain but a thin rim of cytoplasm filled with heavily stained granules, and because of this appearance one might suspect some structural alteration even within normal elements. However, careful study revealed no pathological changes within these cells. Their nuclei were large, well formed and unaltered.

An occasional cell within the nucleus ruber showed a mild fading out of Nissl granules and the presence of an occasional cytoplasmic vacuole. None had any nuclear alterations. The substantia nigra appeared intact.

The vessels within the midbrain were uninvolved. There was no endothelial reaction. No alterations of myelin or of the glial cells were detectable.

Trochlear Nucleus. The large nerve cells of this nucleus were anatomically intact.

Midpons. The motor (masticator nucleus) of the trigeminal nerve, especially on the right side, revealed most extensive and significant alterations (Fig. 4). Most of its cells were shrunken and pyknotic. In many, only small fragments of cytoplasmic material remained, irregularly attached to an apparently normal or shrunken nucleus. In those cells with intact cell bodies, chromatolysis was complete with the cytoplasmic material often being replaced by large vacuoles. The tinctorial properties of some of the cells were partially or completely lost, leaving only a faint outline of the original neuron or resulting in a ghost cell formation. Scattered throughout these severely altered elements were a few with apparently normal architectural arrangement, but even these did not have the appearance of the normal cells, being smaller and containing much less cytoplasmic material. The small vessels within this nucleus showed a moderate degree of endothelial proliferation and a marked vascular congestion. No glial changes were apparent.

The corresponding nucleus on the left side was much less severely involved, although definitely damaged. Here most of the cells showed only swelling and partial chromatolysis. Only scattered elements presented diffuse involvement. In some cells the Nissl substance stained very lightly, while in others it was entirely absent or replaced by intracellular vacuoles. The cell nuclei were usually intact although occasionally swollen and eccentrically placed.

The pontine (sensory) nuclei of the fifth cranial nerve just lateral

to the motor nuclei normally present cells which are somewhat smaller than the motor elements. However, in these sections they appeared larger due to the extensive destruction and shrinkage of the motor cells (Fig. 5). The sensory elements revealed a fairly large, centrally placed nucleus surrounded by a somewhat irregular cell body. Some of the cells were slightly elongated. Their Nissl granules were very fine and stained very lightly although their architectural details were easily made out with higher magnifications. No nuclear changes were visible. These elements were surprisingly healthy, especially when contrasted with the cells of the adjacent motor nucleus. There was a mild congestion but no endothelial proliferation in this nucleus.

The scattered cells of the superior vestibular nuclei were normal. Their cell bodies were distinct and well outlined. The Nissl granules were fairly large and evenly distributed throughout the cell body.

The cells of the pontine nuclei were also well preserved and showed no consistent alterations. Only an occasional cell revealed a slight fading out of some of its tigroid bodies.

Lower Pons. The abducens nuclei were uninvolved. All of the cells were filled with large, deeply stained Nissl granules. The cell nuclei were centrally placed and appeared to be healthy.

The facial nuclei showed very little change (Fig. 6). An occasional cell revealed a mild focal tigrolysis. There was a slight variation in the size of some of the cellular elements but no structural abnormality.

In the vicinity of both the abducens and facial nuclei there was marked congestion but no hemorrhages. No endothelial changes were observed.

The dorsal and ventral cochlear nuclei as well as the lateral vestibular nuclei were for the most part uninvolved. Scattered cells showed very mild changes, involving primarily the tigroid substance. In some the Nissl granules were partially absent, while in others these granules, although not reduced in number, showed a tendency to stain more intensely around the cell periphery, producing the appearance of a perinuclear chromatolysis. In the ventral cochlear nuclei, a few cells showed a slight irregularity and a mild retraction of their processes. No nuclear changes were apparent. Many of the vessels in the vicinity of these nuclei exhibited a mild endothelial swelling. An occasional vessel was completely occluded by endothelial proliferation. Congestion was very intense but no hemorrhage had resulted.

The cells of the spinal tract of the fifth cranial nerve were not distinctly made out. In most sections they were few and very small. Their tigroid granules, although very fine, appeared intact. Their nuclei were often eccentrically placed.

The components of the superior olive were very irregular in shape and size. The larger cells contained deeply stained, evenly distributed Nissl granules about a centrally placed nucleus. An occasional cell showed an early perinuclear tigrolysis. In the smaller cells the Nissl granules were very fine, stained very lightly, and were more difficult to make out; their nuclei were often eccentrically placed.

Upper Medulla. The cells of the solitary fasciculus were small, and irregular in outline. Most of their tigroid substance was very fine and evenly distributed throughout the cytoplasm; a few cells contained heavier, more intensely staining granules interspersed among the finer ones. These heavy granules were evenly distributed and showed no peripheral arrangement. The preservation of structural detail indicated that this cytoplasmic appearance might be normal for these cells. Unquestionable injury, however, occurred to many elements within this nucleus. Such showed an eccentrically placed nucleus which often stained very lightly, having lost some of its tinctorial properties. A few cells contained little or almost no cytoplasmic material, leaving an isolated pyknotic or lightly stained nucleus. Even an occasional swollen ghost cell could be observed. From cursory study it appeared that about one-half of the cells within this nucleus had undergone some type of structural alteration.

Middle Medulla. The dorsal nucleus of the vagus was more severely injured than any other nuclear group in the nervous system. Hardly a normal cell was to be found. The most constant alteration was a perinuclear chromatolysis, with only a thin rim of Nissl granules remaining around the periphery of the cells (Figs. 7 and 8). Many of the cells were swollen, rounded and had lost much of their tinctorial properties. Many of their processes were shrunken, fragmented, or had entirely disappeared. Most of the neurons were small and appeared to have lost considerable cytoplasmic material. The cell nuclei were also altered, many being eccentrically placed, swollen and even pyknotic.

(Sections through this nucleus were also available from a second case of tetanus, the patient dying from an apparent medullary involvement. In this case the dorsal nucleus of the vagus revealed even more advanced and more permanent alterations. Most of its elements were extremely shrunken and pyknotic (Fig. 9). The neurons appeared as small dark masses within which no structural details could be made out. Most of their processes were shortened and narrowed, or were fragmented or entirely absent. In both cases hardly a normal cell was found throughout the nuclei. Congestion was prominent in the vicinity of the nuclei but no endothelial changes were found.)

Nucleus Ambiguus. The cells in this nucleus were not nearly as

severely damaged as were those of the dorsal nucleus. An occasional cell was swollen, its processes being fragmented or entirely absent, giving the cell a rounded appearance. The Nissl granules in these cells were reduced in number and generally were very finely granular, with only a few heavy granules scattered near the periphery of the cell body (Fig. 10). None of the cell nuclei appeared involved.

The large cells of the hypoglossal nuclei, although lying almost adjacent to the greatly damaged dorsal nucleus of the vagus, showed no histological alterations (Fig. 11). All of the architectural details of their elements could be very easily discerned. There was, however, a marked congestion but no endothelial change.

Spinal Cord. The cellular elements within the cord were surprisingly well preserved. Most of the anterior horn cells showed no changes (Fig. 12). Interspersed among these predominantly healthy elements were a few showing a mild fading out of the Nissl granules or even a beginning perinuclear chromatolysis. None of the anterior horn cells showed nuclear changes. The scattered cellular changes were not limited to any particular segment of the cord, but were irregularly and diffusely scattered throughout. There was a mild congestion within the cord but no endothelial change.

DISCUSSION

It is apparent from this study that in certain cases of tetanus the toxin, though distributed throughout the nervous system, appears to have a specific action upon certain cell groups, a selectivity that may correlate the elements of the clinical picture presented by this illness. In the present case there can be no doubt that tetanus toxin showed a selective action upon two cell groups, namely, the motor nucleus of the trigeminal and the dorsal nucleus of the vagus, the involvement of the latter doubtless producing the respiratory failure. From the clinical symptoms in tetanus, it is apparent that the toxin produces functional impairment in almost every case, but in those cases in which the course of the illness is very rapid and of but a few days' duration, insufficient time elapses to allow structural changes to become apparent. When the duration of the disease is sufficiently prolonged, usually beyond the fifth day, visible changes occur within the nervous system. The first elements to become involved are the nerve cells. The distribution of this cellular damage is most irregular and appears to be widely scattered throughout the nervous system. Most of the cellular alterations are mild and consist primarily of swelling and perinuclear chromatolysis. Such changes are reversible and may disappear after recovery from the illness or after neutralization of

the toxin by antitoxin. Within scattered cells or groups of cells there do occur more severe and definitely irreversible changes, some consisting of fragmentation of the cell processes, vacuolization and fragmentation of the cell body, pyknosis of the nuclei, and even pyknosis of the entire cell, or ghost cell formation.

Most of the investigators studying the possible pathological changes in tetanus have limited their studies to the cells within the spinal cord, and their reports have varied from absolutely no alterations (Leyden,² Tauber,³ Vincenzi,⁴ Nageotte and Ettlinger⁵) to most extensive changes (Tauber,³ Sjövall,⁷ Goebel,⁹ Matthes,¹⁰ Elischer,¹¹ von Halban,¹² Barros¹³). Most investigators seem to agree that the chief damage occurs to the motor cells of the cord, and almost every degree of cell damage has been reported. Generally, however, the cell alterations have been mild and widely scattered throughout all segments of the cord with no tendency to localize. Sjövall, from his studies, suggested that the cell changes in tetanus were to be correlated with the tetanic motor irritation and, therefore, occurred on the side and in the region of the cord that supplied the involved limbs. These observations have not been substantiated in the literature or from my studies. In contrast to much of the literature, changes within the cord elements in my cases were strikingly scanty and even those present were mild and of a definitely reversible nature.

Cellular alterations in tetanus, although most frequently studied within the spinal cord, also appear within the rest of the central nervous system. Sjövall,⁷ Elischer,¹¹ von Halban,¹² Minassian,¹⁴ Jukowsky¹⁵ and Ewing¹⁶ have all recorded alterations within the cranial nerve nuclei or within the motor cortex of man and experimental animals. Elischer observed some paling and pyknosis within the cells of the 5th and 7th cranial nerves although the cell architecture was intact. Von Halban described vacuolization and extensive peripheral chromatolysis within the cells of the motor cortex and the 7th and 12th cranial nerve nuclei. Jukowsky described a vascular dilation within the medulla but did not mention any cellular changes. None of these investigators made an attempt to establish the presence of a definite selectivity of the tetanus toxin to certain cranial nerve nuclei. From my histological studies it seems evident that in certain cases of tetanus the toxin does have a specific affinity for certain cranial nerve nuclei, producing extensive and permanent cellular damage. The specific selectivity of the motor nucleus of the 5th cranial nerve and the dorsal nucleus of the vagus is most interesting when one considers the close correlation of these pathological alterations to the clinical symptoms presented by the patient.

Death in tetanus has generally been assumed to result from three causes; namely, exhaustion, circulatory failure from the great demand made upon the heart, and, finally, asphyxia due to spasm of the glottis, diaphragm and intercostal muscles. Death from medullary involvement has not been given very serious consideration. It is difficult to observe such specific focal damage to important medullary nuclei, as has been demonstrated in the foregoing case, without concluding that certain cases of this disease must terminate fatally due to severe damage to these centers with resultant cardiac or respiratory failure. Such a conclusion can be verified clinically when one observes cases in which, in spite of the absence of severe convulsions or in spite of adequate medicinal control, the patient continues to show marked cardiac and respiratory irregularities and finally dies of an apparent respiratory failure. The possibility of such cerebral involvement in tetanus must force one to be more guarded in his prognosis of this illness even in the face of apparent improvement and medical control.

CONCLUSIONS

1. Detailed pathological studies were undertaken in a case of tetanus showing definite signs of medullary involvement throughout the course of the illness.

2. Definite changes were observed within certain selected areas. The most extensive involvement occurred within the motor nuclei of the 5th and the dorsal nuclei of the 10th cranial nerves. These nuclei showed alterations within almost every nerve cell, the changes often being of a permanent and irreversible nature. The rest of the nervous system presented but scattered and usually minor changes.

3. The selectivity of the tetanus toxin for specific cranial nerve nuclei suggests the possibility that some deaths in this disease may be due to medullary involvement, with resulting respiratory or cardiac failure.

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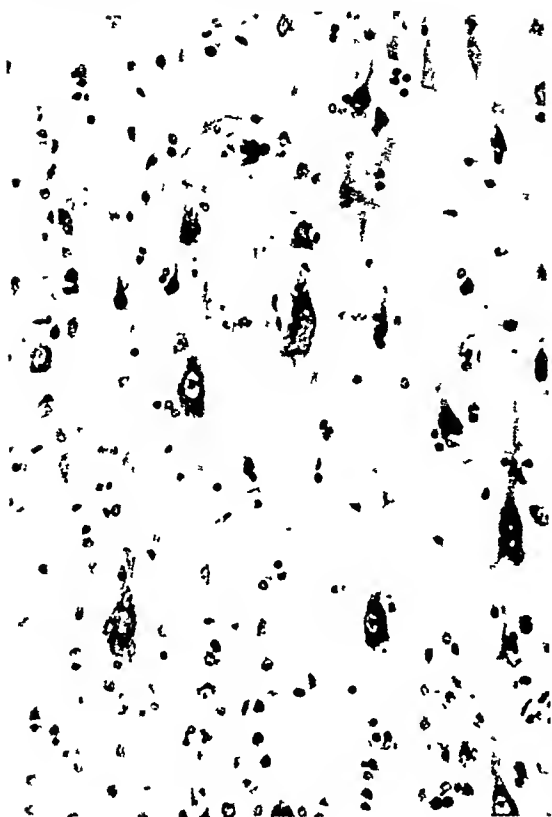
[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 84

- FIG. 1. Motor cortex. The cells appear normal. There is no apparent increase in the interstitial elements. Nissl stain. $\times 150$.
- FIG. 2. Motor cortex. High-power photomicrograph through the area in Figure 1, demonstrating in more detail the normal cyto-architecture of the nerve cells. Nissl stain. $\times 350$.
- FIG. 3. Oculomotor nucleus. The nerve cells as well as the interstitial elements show no alterations. Nissl stain. $\times 275$.
- FIG. 4. Motor nucleus of the 5th cranial nerve. The cells show extensive damage consisting of almost complete chromatolysis, disappearance of the cell processes, shrinkage of the cell body, fragmentation and pyknosis. Even the nuclei have been damaged in some of the neurons. Nissl stain. $\times 275$.

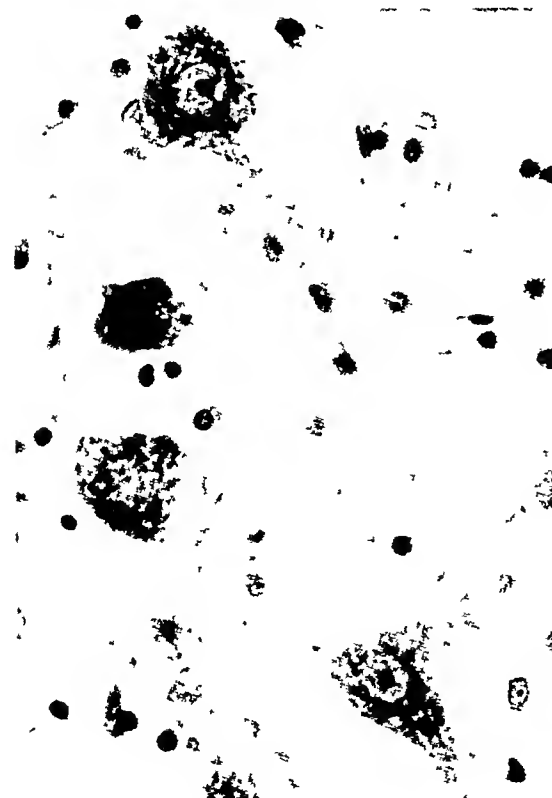
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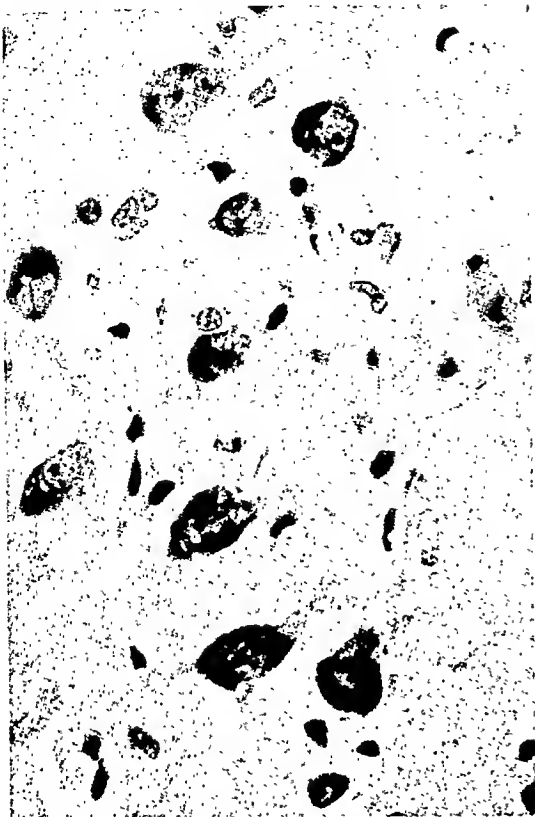
Baker

Medullary Involvement in Tetanus

PLATE 85

- FIG. 5. Sensory nucleus of the 5th cranial nerve. These cells, in contrast to the motor elements, are uninvolved. Their architectural details can easily be made out. Nissl stain. $\times 275$.
- FIG. 6. Facial nucleus. The nerve cells are intact. A single neuron in the lower portion of the field contains a large cytoplasmic vacuole and shows a mild chromatolysis. Nissl stain. $\times 150$.
- FIG. 7. Dorsal nucleus of the vagus nerve. This control section was taken from an apparently normal nucleus. These cellular elements may be compared with those in Figures 8 and 9 which were taken from cases of tetanus. Nissl stain. $\times 275$.
- FIG. 8. Dorsal nucleus of the vagus nerve. There is characteristic perinuclear chromatolysis in almost all the cells. The cell processes have been partially destroyed, but the nuclei appear uninvolved. Nissl stain. $\times 275$.

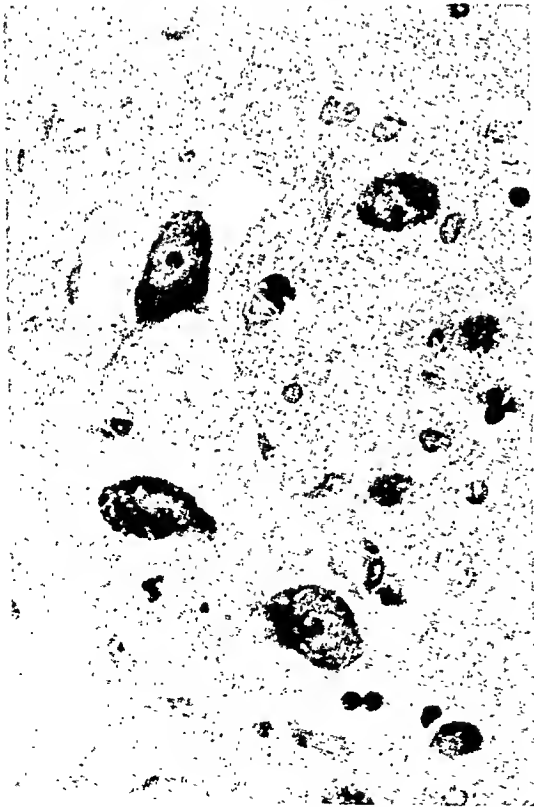
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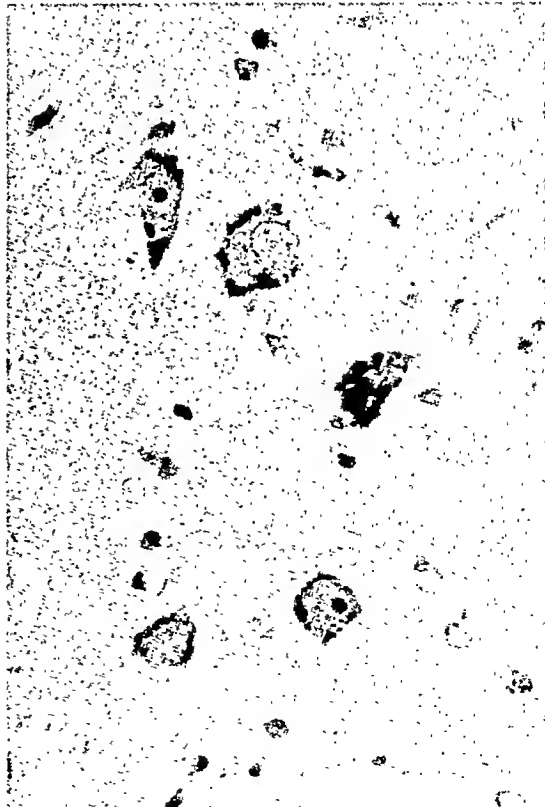
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Baker

Medullary Involvement in Tetanus

PLATE 86

FIG. 9. Dorsal nucleus of the vagus nerve. This photomicrograph demonstrates a more advanced stage of the cellular damage. The neurons are shrunken and pyknotic. These changes are no doubt of an irreversible nature. Nissl stain. $\times 275$.

FIG. 10. Nucleus ambiguus of the vagus nerve. There is early tigrolysis and beginning cytoplasmic shrinkage. Some of the cell processes have disappeared. Nissl stain. $\times 275$.

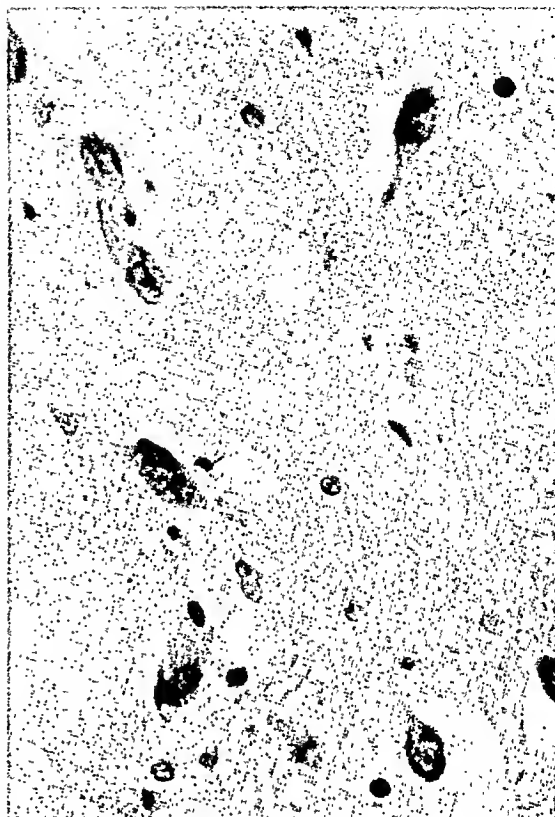
FIG. 11. Hypoglossal nucleus. These cells are uninvolved even though they are situated very close to the severely damaged dorsal nucleus of the vagus. Nissl stain. $\times 275$.

FIG. 12. Spinal cord. This photomicrograph demonstrates the anterior horn cells. Most of the cells are intact, although an occasional one does show a mild diffuse or even perinuclear chromatolysis. Nissl stain. $\times 275$.

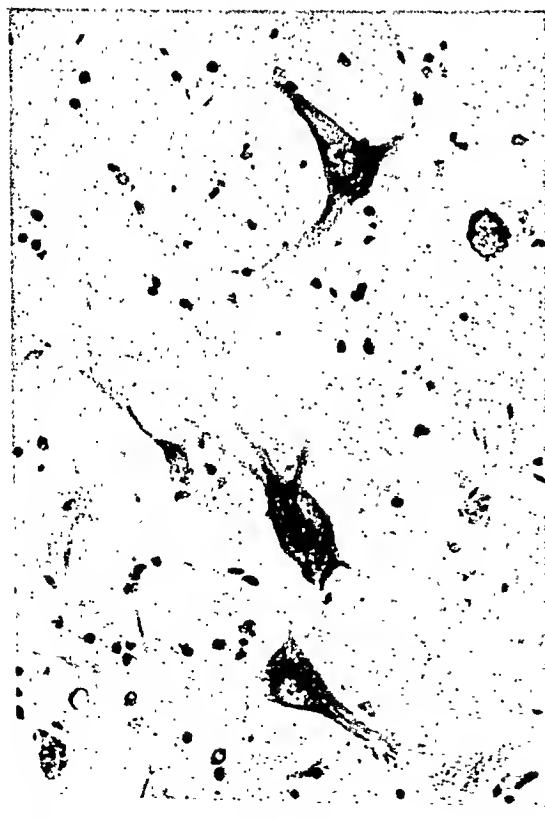
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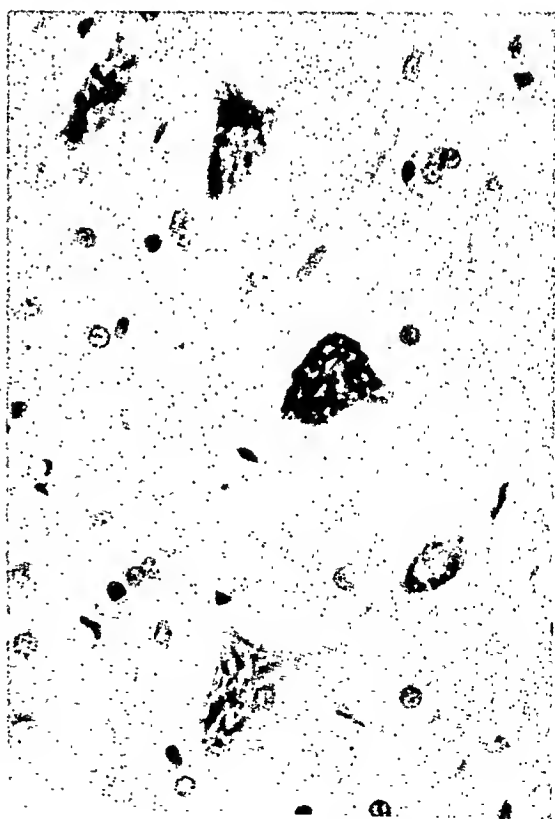
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Baker

Medullary Involvement in Tetanus



TUBERCULOSIS OF THE TONSILS *

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Disagreement exists as to the mode by which the tubercle bacillus infects the faucial tonsils. Weller ¹ studied 204 cases of active tonsillar tuberculosis and came to the conclusion that in 90 per cent of the cases the infection had arisen in the crypts. Usually the infection was unilateral and there was no obvious tuberculosis elsewhere in his patients. He described 16 cases in which the tubercles were located in the follicles, often in the germinal centers. Most of these were bilateral infections. Weller decided that the cryptal type of infection was primary (in the sense of exogenous) and the follicular type a secondary, blood-borne infection. The views of this investigator, based as they are on the histology of a large number of lesions, seem well grounded. That they are not universally accepted is shown by the statement of Hansel ² that "both direct and indirect evidence make it probable that the tonsils are usually infected by the blood stream." Libin and Travushkina ³ stated that the difficulty of making a clinical diagnosis of tonsillar tuberculosis exists because the pathological process begins in the depths of the tonsil and that this origin is in favor of a blood stream infection. This last seems to be an unwarranted deduction since the crypts extend deeply into the tonsil and an infection originating at the base of a crypt would originate in the depths of the tonsil.

Schlittler ⁴ had previously examined 48 tonsils in a series of 89 cases of tuberculous lymphadenitis. He decided that these were examples of primary tuberculosis of the tonsil because (1) 73 per cent were unilateral, (2) 63 per cent of the patients were under the age of 12, (3) sputogenic infection seemed contraindicated by the clinical histories. Later, he reconsidered the facts after examining the tonsils in another series of 41 cases of tuberculous lymphadenitis. In this group most of the patients were over 12 years and the percentage of unilateral infection was lower. His final conclusion was that the majority of the cases were not primary but either post-primary or secondary hematogenic. The differences of opinion which exist have been commented on by Long, Seibert and Gonzalez ⁵ and the literature in this connection reviewed. They stated that Schlittler believes most infections to be hematogenous, but this overlooks Schlittler's post-primary group. The pathogenesis of tonsillar tuberculosis in cases of pulmonary disease chiefly interested Long and his associates. They

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studied a considerable number of cases and found that in open tuberculosis of the lungs the tonsillar infection was frequently bilateral and apparently originated in the crypts. There was nothing to support the hematogenic mode of infection. This is in agreement with the ideas of Weller.¹ The clinicopathological data on the cases studied in this present paper support Weller's views.

MATERIAL AND METHODS

In the 19,000 tonsillectomies done at the Henry Ford Hospital from 1922 to 1941 inclusive, there have been 35 cases of active tonsillar tuberculosis. At this clinic all tonsils are examined microscopically. A single section is cut at right angles to the mucosa through the long axis of the tonsil. The diagnosis of tuberculosis was made in those cases which showed typical active tubercles. All available clinical and laboratory data were collected and the morphological picture of the diseased tonsils was reviewed in an attempt to establish the pathogenesis for this series.

In this study the initial assumptions were that a cryptal infection is due to the passage of the tubercle bacillus through the mucosa (or possibly through ulcerations at the bases of the crypts); that tubercles found in relation to the follicles and germinal centers in the absence of relation to the crypts signify a hematogenous spread to the tonsil; that lymphatic spread can be ignored because the tonsil has no afferent lymphatics and retrograde spread is unlikely. The results of the study were not such as to require alteration of the assumptions. The following classification was used:

1. Unilateral
 - A. Cryptal
 - (1) Primary (by inhalation or ingestion)
 - (2) Post-primary (by inhalation, ingestion, or sputum)
 - B. Follicular
2. Bilateral
 - A. Cryptal
 - (1) Primary
 - (2) Post-primary
 - B. Follicular

The number of cases in each category and the distribution of the cases by serial numbers are shown in Table I.

UNILATERAL INFECTIONS WITHOUT PULMONARY TUBERCULOSIS

Case 1. A child, 3 years old, was admitted with a history of frequent bouts of upper respiratory infection since the age of 2. No exposure to tuberculosis known. X-ray studies showed hilar tuberculosis and Pott's disease of the seventh dorsal vertebra. Tonsillectomy disclosed a unilateral cryptal infection.

Case 5. A girl, 13 years old, was admitted with an enlarged node on the right side of the neck of 3 years' duration. There had been a sudden increase in size 3 months previously. No history of tuberculosis. Tonsils were enlarged and scarred. The child had been examined previously at another hospital and all findings, including roentgenograms of the chest, were negative. There was a nontender, non-fluctuant node on the right side of the neck, about the size of a hickory nut. Tonsillectomy was done on November 9, 1931. There was a unilateral cryptal tuberculosis. The patient was seen again in 1941, at which time she seemed to be in good health.

TABLE I
Distribution of Cases According to Subdivisions of the Classification

Classification*	Number of cases	Case numbers†
I.	27	1, 5, 6, 7, 8, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35
I.A.	20	1, 5, 6, 7, 8, 11, 12, 13, 14, 15, 18, 20, 23, 24, 25, 26, 28, 29, 30, 31
I.A.(1)	15	1, 5, 6, 7, 8, 11, 12, 14, 18, 23, 24, 25, 29, 30, 31
I.A.(2)	5	13, 15, 20, 26, 28
I.B.	None	None
2.	8	2, 3, 4, 9, 10, 16, 22, 32
2.A.	6	3, 9, 10, 16, 22, 32
2.A.(1)	3	3, 9, 32
2.A.(2)	2	10, 16
2.B.	2	2, 4

* The notations under this heading refer to the classification given in the text.

† Of the 27 cases of unilateral tuberculosis only 20 can be completely classified. The remaining 7 are cases 17, 19, 21, 27, 33, 34 and 35. Whether cases 17, 19, 27, 33 and 35 were cryptal or follicular could not be determined. Both tonsils were removed in case 21, but only one reached the laboratory. Case 34 is probably I.A.(1) but the clinical information is incomplete. One of the bilateral infections, case 22, could not be completely classified because of lack of evidence. Probably it falls under 2.A.(1).

Case 6. A child, 6 years old, was admitted for a routine tonsillectomy. There was no history of exposure to tuberculosis. Tonsillectomy was done on May 22, 1929. There was a mild unilateral cryptal infection. Roentgenograms of the chest on June 24, 1929, were negative; those of June 28, 1930, showed enlarged hilar nodes. On September 17, 1932, roentgenograms of the chest showed a widened hilar shadow but no calcified nodes; those of November 4, 1933, were similar, and on October 19, 1935, a tubercle of Ghon was seen in the right fourth interspace. Throughout this time the child remained in good health.

Case 7. A child, 9 years old, was brought in at the request of school authorities for a routine tonsillectomy and adenoidectomy. Physical examination was essentially negative and the lungs were stated to be negative. The tonsils were enlarged and the anterior cervical nodes palpable. Tonsillectomy was done on January 19, 1931. Microscopically there was a cryptal infection in one tonsil. The other was uninvolved. The child was not seen again at this clinic.

Case 8. A white girl, 18 years old, was first seen on February 20, 1926, complaining of large nodes at the angle of the jaw on the left side of 4 months' duration. Physical examination was essentially negative. Tonsillectomy on February 23, 1926, disclosed an extensive cryptal infection in one gland. Shortly afterward she developed a draining cold abscess on the left side of the neck. In 1938 she was in fairly good health and the infection was quiescent. There was never any evidence of pulmonary tuberculosis.

Case 11. A white boy, 27 months old, was brought in because of cervical adenitis of 3 weeks' duration. An uncle had osseous tuberculosis. A brother, 10 months of age, had died of "mucous colitis" a few weeks previously. The child showed an enlargement of the entire group of right cervical lymph nodes without redness, pain, or fluctuation. Tonsillectomy on April 4, 1927, disclosed a unilateral cryptal infection. Roentgenograms on May 3, 1927, were read as showing no hilar enlargement or parenchymal involvement. On March 9, 1928, one of the nodes on the right side of the neck was incised and a tuberculous abscess found. Roentgenograms on January 24, 1929, showed hilar enlargement but no definite tuberculosis.

Case 12. A white girl, 6 years old, was brought in for routine tonsillectomy. The history and examination were negative. Tonsillectomy on October 23, 1926, disclosed a mild unilateral cryptal infection. Roentgenograms on November 4, 1926, showed a calcified node in the neck on the right side, as well as some calcified hilar nodes.

Case 14. A white boy, 5 years old, was brought in because his father thought that he should have his tonsils removed. He had always been healthy. General physical examination was negative. Tonsillectomy was done on August 18, 1927. Microscopically there was a unilateral cryptal tuberculosis. The child was seen again for a recheck on September 14, 1927, and the lungs were stated to be negative.

Case 18. A child, 5 years old, was admitted whose mother had shown a positive sputum on several occasions. Roentgenograms on December 13, 1934, showed calcification in the right hilus and possibly in the parenchyma on the right. Tonsillectomy on January 3, 1935, disclosed a severe localized unilateral infection. This child was last seen in 1941, at which time there were areas of calcification in the hilus but no evidence of parenchymal disease.

Case 23. A white girl, 20 years old, came in with the complaints of bronchitis, chronic cough and lack of energy. Physical examination was essentially negative except for deflected septum and hypertrophic tonsils. Tonsillectomy was done on November 16, 1929. Microscopically there was a unilateral cryptal infection. Some of the tubercles had a little central necrosis but there was no caseation. The patient did not return for a recheck.

Case 24. A boy, 4 years old, was admitted whose father had pulmonary tuberculosis. On December 7, 1931, the child gave a positive reaction to 1/10 mg. O.T. A roentgenogram made on the same day showed hilar accentuation which was not definitely tuberculous. Tonsillectomy was done on May 6, 1932. There was a mild unilateral cryptal infection. On March 10, 1933, a cold abscess ruptured in the right groin.

Case 25. A white man, 27 years old, was admitted for pain in the back and stomach. Tonsillectomy was done in 1929 and a unilateral cryptal infection was found. He was not seen again until 1937; roentgenograms taken at that time showed calcified nodes in the left hilar area. There was no clinical tuberculosis.

Case 27. A child, 5 years old, was admitted who was poorly developed and nourished. Her mother had died with pulmonary tuberculosis. Physical examination showed large nodes at the angles of the jaw but the lungs appeared clear. The child had a positive tuberculin reaction, and roentgenograms showed increased hilar density. Tonsillectomy was done on July 23, 1928. Microscopically a proliferative tuberculous lesion was present in one tonsil but a definite relation to either crypts or follicles could not be established.

Case 29. A girl, 6 years old, was brought in because of a limp in the right leg of 2 weeks' duration. There was restricted rotation and adductor spasm. The tonsils and cervical lymph nodes were enlarged. She was treated for tuberculosis of the hip. On April 11, 1939, tonsillectomy showed a unilateral cryptal tuberculous infection. Several roentgenograms showed hilar enlargement but no parenchymatous lesions of the lungs.

Case 30. A white boy, 3½ years old, was admitted who had been followed in the clinic since the age of 6 weeks. No exposure to tuberculosis was known. There had been frequent attacks of otitis media. On February 21, 1934, tonsillectomy was done. There was a unilateral cryptal infection. Roentgenograms on May 23, 1934, showed hilar accentuation; those on November 27, 1936, showed a similar appearance.

Case 31. A boy, 8 years old, was admitted whose mother had active pulmonary tuberculosis. She brought the boy into the hospital because she thought that he had chickenpox. The lesions were insect bites, however. Tonsillectomy was done on December 9, 1929. There was a unilateral cryptal infection. Roentgenograms on February 10, 1930, showed calcified nodes in the right hilus; those on January 13, 1941, showed a similar picture.

Case 33. Tonsillectomy was done on a white male, 22 years old, on March 29, 1924. Microscopically one of the tonsils showed a proliferative tuberculous lesion, but it could not be related definitely to crypts or follicles due to the way the section was cut. Patient was called in for a recheck. The chest was found to be negative. Roentgenograms were advised but the patient refused.

Case 34. A patient, 31 years old, came in with complaints of stomach ulcer. There was no family history or past history of tuberculosis. The lungs were negative; tonsils were slightly enlarged, and cervical lymph nodes were palpable. Tonsillectomy was done on January 31, 1930. Microscopically there was a unilateral cryptal infection. No further studies were carried out.

Discussion

Cases 1, 5, 6, 7, 8, 11, 12, 14, 18, 23, 24, 29, 30 and 31 are most easily interpreted as primary tuberculosis of the tonsils. In some instances the available data suggest the possibility of bovine organisms as the infecting agents. Case 25 is less clear owing to the age of the patient. Data on cases 27, 33 and 34 are incomplete.

UNILATERAL INFECTIONS WITH PULMONARY TUBERCULOSIS

Case 13. A white woman, 58 years old, was admitted complaining of bone and joint pain. Many years ago she had had an attack of pleurisy. Tonsillectomy was done on May 10, 1927. There was extensive scarring of both tonsils with unilateral cryptal tuberculosis. Patient was called in for a recheck and on June 14, 1927, roentgenograms showed thickening and infiltration of the right apex, probably tuberculous.

Case 15. A white woman, 36 years old, was admitted complaining of chronic backache. Tonsillectomy was done on December 1, 1927. There was a unilateral cryptal infection. Roentgenograms of the lungs on December 16, 1927, showed calcified hilar nodes and probable cavity at the right apex. There were râles in the right apex posteriorly. Treatment was undertaken at home. Roentgenograms on May 7, 1937, showed inactive parenchymal lesions in the right apex.

Case 17. A white man, 25 years old, was first seen on April 29, 1924, with the complaint of hoarseness of 2 months' duration. This had followed an attack of tonsillitis or sore throat. Physical examination was essentially negative except for slight enlargement of the cervical lymph nodes. Pus could be expressed from the upper pole of the right tonsil. Tonsillectomy was done on May 5, 1924. Microscopically there was a unilateral infection consisting of one tubercle with a necrotic, partially caseous center. The specimen was too distorted to determine whether it was related to the crypts. The tonsils were extensively scarred. Re-examination of the chest showed signs of active tuberculosis, and roentgenograms on May 23, 1924,

showed an extensive bilateral parenchymatous infiltration, with a large cavity on the left side. The patient was not seen again.

Case 19. A white man, 29 years old, came in complaining of sore throat and frequent attacks of tonsillitis. There was no history of tuberculosis in the family. Physical examination showed a partial stricture of the nasopharynx but no positive findings in the lungs. Tonsillectomy and plastic operation were done on January 3, 1924. Microscopical examination showed a diffuse epithelioid reaction with giant cells in one tonsil, without caseation or necrosis. The section did not include any crypts. A relation to germinal follicles could not be made out. The tissue from the nasopharynx showed extensive productive tuberculosis. Roentgenograms in 1926 showed evidence of apical pleuritis with thickened pleura at the right base.

Case 20. A white boy, 8 years old, was admitted with no history of exposure to tuberculosis. Tonsillectomy was done on April 20, 1927, following which he did not do well. There was a unilateral cryptal infection. On May 3, 1927, a few fine râles were heard in the left interscapular region. Roentgenograms on May 5, 1927, showed an area of consolidation in the midportion of the left lung with hilar enlargement and infiltration of both upper lobes.

Case 21. A white girl, 5 years old, had been followed since early infancy. A roentgenogram taken on August 30, 1930, showed a small but definite amount of fibrosis in the right upper lobe. Tonsillectomy was done on September 9, 1930. Both tonsils were removed but only one reached the laboratory. This showed a localized cryptal infection. Roentgenograms on September 28, 1931, showed some accentuation of the hilar areas and an area of increased density in the right upper lobe which appeared to be tuberculous.

Case 26. A white man, 37 years old, was referred to the hospital for tonsillectomy. Operation was done on March 25, 1937. There was a unilateral cryptal infection. Roentgenograms on November 9, 1937, showed multiple lesions in both upper lobes. The sputum was positive for tubercle bacilli and there was an early laryngeal tuberculosis.

Case 28. A white female, 24 years old, was given a routine physical examination on February 10, 1931, which disclosed no abnormalities. Tonsillectomy was done on December 22, 1933, and unilateral cryptal tuberculosis was found. She was called in for a recheck and gave a history of fatigue and loss of weight. Roentgenograms on January 26, 1934, showed bilateral parenchymatous lesions with a probable cavity on the left side.

Case 35. A white man, 20 years old, was a known case of pulmonary tuberculosis. Tonsillectomy on November 3, 1923, showed a unilateral infection, but due to the poorness of the section the morphological type could not be diagnosed. This patient was known to have sputum positive for tubercle bacilli.

Discussion

Four of these cases were active and probably open, so the sputogenic origin of the tonsillar lesion seems fairly certain. Case 13 was apparently neither active nor open. Assuming this to be true, it must have been a post-primary exogenous infection. This should not seem strange, in view of the ubiquity of the organism and the frequency of tonsillar erosions. From the scarred appearance of the tonsils in this case it might be guessed that there had been previous infections, some of which could have been sputogenic. Data on cases 17, 19, 21 and 35 are incomplete.

BILATERAL CRYPTAL INFECTIONS

Case 3. A colored child, 14 months old, was nursed for a short time by his mother who died of pulmonary tuberculosis 3 months after his birth. There were enlarged cervical, inguinal and axillary lymph nodes. Tonsils were enlarged. Roentgenograms on June 30, 1931, showed enlarged hilar nodes. Tonsillectomy on September 30, 1931, showed a bilateral cryptal tuberculosis.

Case 9. A white woman, 24 years old, complained of an ache in the upper chest and shoulders following an attack of tonsillitis. Physical examination was essentially negative except for chronic tonsillitis and enlarged cervical nodes. Tonsillectomy was done on August 15, 1924. Microscopically there was a widespread bilateral infection arising in the crypts. The patient was called back for a recheck but no evidence of extratonsillar tuberculosis was found. However, roentgenograms were not made.

Case 10. A white man, 30 years old, complained of a "run-down" feeling, and pain below the shoulder blade on the right side. There was impairment to percussion in the same region. Roentgenograms showed an extensive, radiographically inactive, infiltration of both upper lobes. Tonsillectomy was done on October 1, 1925. There was a bilateral cryptal infection.

Case 16. A white male, 29 years old, complained of chills, fever and night sweats over a period of 6 months. There were moist râles in the left interscapular region. Roentgenograms on February 16, 1929, showed active parenchymatous changes in the right upper and middle lobes. Tonsillectomy was done on March 4, 1929. There was a heavy infection arising in the crypts of both tonsils. The patient died elsewhere a few months later.

Case 22. A boy, 12 years old, was brought in for a routine tonsillectomy. There was no family or personal history of tuberculosis. Physical examination was negative except for large and injected tonsils and palpable cervical nodes. Tonsillectomy was done on March 19, 1928. There was a bilateral cryptal infection with much scarring. The patient did not return for a recheck.

Case 32. A white woman, 22 years old, had been followed because of a gynecological complaint for about 1 year when an examination of the chest showed an enlargement of the retrosternal dullness. On March 20, 1925, roentgenograms showed a large rounded nodule in the right hilus. The impression was lymphosarcoma. She was given x-ray therapy and roentgenograms on May 29, 1925, showed that the circular shadow had almost disappeared. On November 16, 1925, she had a severe attack of tonsillitis. About 3 months later a huge cervical lymph node had developed. There was no general glandular enlargement. Specimen taken for biopsy of this node showed tuberculosis. Tonsillectomy on May 20, 1926, showed a massive bilateral infection clearly arising in the tonsillar crypts. Following the excision for biopsy a draining sinus developed which persisted for many years.

Discussion

Case 9 appears to be the result of a heavy infection by the oral route, which could have come from infected milk or some similar source. Case 32 also seems to be an oral infection, interesting because of the severe degree of lymphadenopathy, and similar in many respects to case 3. Because of lack of evidence, case 22 cannot be commented upon. Some or all of these four cases may have been due to a bovine type of infecting organism. Mitchell ^{6, 7} isolated the bovine organism 65 times and the human organism 7 times in a series of tuberculous

cervical nodes. In 26 cases of tuberculous tonsils he found the bovine type 20 times. Case 16 is clearly, and case 10 probably, of sputogenic origin. Long, Seibert and Gonzalez,⁵ in the study of 81 cases of tonsillar tuberculosis, secured roentgenograms on 35 patients and found active pulmonary tuberculosis in 18 (16 of the adult type, 2 of the childhood type). The infection in these tonsils appeared to originate in the crypts. However, Long and his associates had a small series of "closed" cases where the histological picture was similar to that seen in the "open" cases. The mechanism of infection was uncertain. Possibly they may have been post-primary infections with no relation to the pulmonary lesions.

BILATERAL FOLLICULAR INFECTIONS

Case 2. A white male, 33 years old, was first seen on December 24, 1932, 1 month after an attack of sudden weakness, chilliness and sweating. Following this he continued to feel weak and tired, ran an irregular fever and lost 11 lbs. Physical examination was essentially negative. The tonsils were small. Innumerable laboratory studies were done without finding the source of the trouble. Tonsillectomy was done on January 11, 1933. He left the hospital and, in another city, shortly developed a severe sloughing infection of the tonsillar beds, necessitating hospitalization for several days. The patient was not seen again at the hospital, but in 1939 he wrote asking that his clinical history be forwarded to the physician who was treating him for a "recurrence of his trouble." The tonsils in this case showed many small tubercles located near, and in some cases in, the germinal follicles. There was no apparent relation to the crypts.

Case 4. A white boy, 4 years old, was first seen on June 22, 1931, with complaint of loss of appetite and persistent pain on the left side of the chest. An uncle, who had lived with the family in constant contact with the patient, had died recently of advanced pulmonary tuberculosis. On admission to the Henry Ford Hospital the tonsils and cervical nodes were moderately enlarged. A few fine râles were heard over both upper lobes. Roentgenograms on June 30, 1931, showed accentuated hilar areas, enlarged nodes in the left hilus and suggestive evidence of a cavity at the left base; those on August 4, 1931, showed evidence of consolidation at the left base. Tonsillectomy was done on March 16, 1932. There was a bilateral infection with hard tubercles located near and in the germinal centers. In some areas there was a nonspecific follicular necrosis. Roentgenograms on July 8, 1932, showed no definite parenchymatous changes although there was suspicious infiltration of the hilar areas.

Discussion

The history in case 2 is quite compatible with the histopathological finding of a follicular infection. In case 4 conditions favorable for a sputogenic infection may have been present, yet blood stream infection took place instead. Under similar conditions a mixed cryptal and follicular infection might occur.

GENERAL DISCUSSION AND SUMMARY

Relation of Tonsillar Tuberculosis to Tuberculosis of Cervical Nodes. Blatt and Greengard⁸ stated that whereas some authorities believe tuberculosis of the cervical nodes to be almost invariably due

to bovine organisms with the tonsil as the portal of entry, they, on the contrary, lean to the belief that it occurs most commonly as a result of primary aerogenous infection of the lungs with secondary involvement of the intrathoracic nodes, followed by direct extension to the deep and superficial cervical nodes. Unless I carry the process one step further and assume that the tonsils are infected through the cervical nodes, it would be difficult to explain my cases. Such a retrograde lymphatic infection of the tonsil seems unlikely. Case 8 clearly suggests a sequence of infection from the tonsil to the cervical nodes, as do cases 11 and 5.

Tonsillar Tuberculosis and Hilar Tuberculosis. On the other hand it is possible that the mechanism suggested by Blatt and Greengard⁸ takes place in reverse, so that the infection may travel from the tonsils to the cervical nodes, thence to the hilar and even to the retroperitoneal nodes. Cases 3, 12, 18, 24, 31 and 32 are in point here.

Tonsillar Tuberculosis and "Open" Pulmonary Tuberculosis. It would appear highly probable that most tonsillar infections in this group are sputogenic. A few cases of hematogenic infection will occur also. When the pulmonary lesion is "closed" and the pathological picture in the tonsil is that of a cryptal infection, the dilemma is resolved by postulating a post-primary exogenous infection of the tonsil independent of the lesion in the lung, or a reawakening of a slumbering infection incurred at a time when the pulmonary lesion was temporarily "open."

Hematogenic Tuberculosis of the Tonsils. Weller¹ found 16 of 204 cases to be of this type. In this series there are 2 out of 35 (or 28, if the doubtful cases are omitted). Apparently cases of hematogenic infection are considerably in the minority.

Primary, Post-primary and Hematogenic Infections. In this series, 18 cases (1, 3, 5, 6, 7, 8, 9, 11, 12, 14, 18, 23, 24, 25, 29, 30, 31, 32) have been interpreted as primary; 7 cases (10, 13, 15, 16, 20, 26, 28) as post-primary sputogenic; 2 cases (2, 4) as follicular. The remainder are more or less uncertain for various reasons.

Pathogenesis of Tonsillar Tuberculosis. Cryptal infections are due to the passage of the bacillus through the intact or eroded mucosa of the base of the crypt. Tubercles related to the follicles and germinal centers are hematogenous. Lymphatic spread can be ignored because the tonsil has no afferent lymphatics and retrograde lymphatic spread is unlikely.

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EARLY LÉSIONS OF EXPERIMENTAL ENDOCARDITIS LENTA *

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The transmission of infection with *Streptococcus viridans* by intravenous injection of pure cultures into animals with the production of an experimental disease closely resembling the human endocarditis due to infection with this organism has been previously reported.¹ By the simple procedure of intravenous inoculation one may cause endocardial vegetations in various animals such as rabbits, rats and mice. Rabbits, because of their conveniently large ear veins, have been used by us for the most part. It has seemed best to designate this experimental infectious disease, which may be regarded as specific in the bacteriologic sense, as experimental endocarditis lenta. Our collection of material from these experimental animals has been studied in some detail in order to follow the sequence of events in the experimental disease. Photographs of the fully developed valvular vegetation were shown in the previous paper. In this present communication we purpose to deal with the changes observed in the heart in the earlier stages of the experimental disease, where the lesions are, for the most part, so minute as to be readily overlooked in gross inspection of the heart.

Rabbit 36 was inoculated by intravenous injection of 2 to 4 cc. of a culture suspension of *Streptococcus viridans*, strain P, that had been centrifugalized, washed in saline solution, recentrifugalized and suspended in saline solution, daily on May 10, 11, 12 and 13, 1939. The rabbit died early on May 15th. At autopsy there were visible many small renal abscesses and small rough spots on the tricuspid valve, thought to be early vegetations. Sections through this region actually showed definite early vegetations near the margin of one of the tri-

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cuspid flaps. The earliest changes, however, were seen in the endocardial endothelium of the ventricle and in the myocardial capillaries. Figure 1 represents a small portion of the right ventricle and Figure 3 is a drawing of a small area of this section. In almost every microscopic field one can find streptococci contained within endocardial endothelial cells. Bacteria are few and the endothelial cells seem in many instances to be free from other alteration. Some of them, however, are thickened and partly loosened. Sometimes the nucleus appears more rounded than normally or may even exhibit irregular fragmentation of the chromatin. At one place about midway in the figure there is some material adherent to the endocardium and in this a few erythrocytes can be distinguished. The subjacent myocardium appears unaltered, but in it near the center of the drawing there is a capillary almost filled with a thrombus, containing many streptococci, most of them within polynuclear leukocytes. There is a slight excess of wandering cells external to the capillary wall and, at a short distance to the left in the drawing, there are extravasated erythrocytes. At the bottom of the drawing there are represented four serial sections of this thrombosed capillary, the third in the series belonging to the section represented in the main drawing. Several normal capillaries may be seen, two fairly large ones at the upper right.

Rabbit 34 was inoculated by intravenous injection of 2 to 4 cc. of *Streptococcus viridans*, strain P, that had been washed and then suspended in saline solution, daily on May 10, 11 and 12, 1939. Blood culture taken on May 15th was positive. The rabbit died on the morning of May 16th. At necropsy there was seen a small irregular thickening near the margin of a mitral flap. Sections through this leaflet actually revealed an irregular accumulation of fibrin near the free edge and there were many well stained cocci contained in this fibrin. On examining the section of the leaflet nearer to its base there was seen on the auricular surface an irregular displacement of the endothelial cells in which a few streptococci could be distinguished even in the microscopic section stained with hematoxylin and eosin (Fig. 4). Erythrocytes also were recognized along with amorphous material, apparently fibrin and platelets, adherent to this auricular endothelial surface. Another section of this same mitral flap, at some distance from the first section, stained by the method of Brown and Brenn,² revealed the streptococci more distinctly (Fig. 7). Here also there was distortion of the endothelial cells and of their nuclei, and a small thrombus, containing several erythrocytes, was adherent over an area almost 0.1 mm. in extent. In both drawings the subendothelial edema is distinctly shown and it is worthy of note that the endothelium

on the ventricular surface seems to have escaped alteration to a very large extent. Careful study of several sections failed to disclose any cocci included by phagocytosis in the endothelium on the ventricular surface of the mitral leaflet.

Rabbit 33, weighing 2,000 gm., was inoculated by intravenous injection of 2 cc. of culture no. 353 daily on May 10, 11, 12 and 13, 1939. There was no further inoculation. Blood culture taken on May 15th gave positive growth, as also did a second one taken on May 18th. The animal died at 2:30 p.m., May 19th, or 9 days after the initial inoculation. At autopsy there were gross infarcts in both kidneys and minute vegetations were recognized on the mural endocardium of the right ventricle and of the left ventricle as well as on the mitral and aortic valves. The liver showed considerable coccidiosis.

Figure 2 shows a photomicrograph at low magnification representing a section passing through the left ventricle, an aortic cusp and a portion of the aortic wall. The finer details are illustrated by the colored drawings. In Figure 5 there is pictured a small bit of the ventricular endocardium. The endothelial cells contain engulfed streptococci but seem not to be greatly altered. A capillary just beneath the endocardium is cut longitudinally and appears to be normal. The myocardial cells, however, are separated by edema fluid and are also somewhat fragmented. The endothelial lining of the ascending aorta, shown in Figure 8, also contains many cocci and, for the most part, is without marked alteration of the endothelium or the subjacent stroma. However, over an area of about 0.5 mm. in length the endothelial cells have been destroyed by the massive proliferation of the bacteria and the underlying stroma has been invaded by streptococci with the production of edema and, in places, coagulation necrosis. Over these latter, more severe lesions, the bacteria are seen as compact masses of cocci directly in contact with the fluid within the aortic lumen.

A photomicrograph of the section of the aortic cusp is shown in Figure 10 and more detail is shown in the colored drawings. Figure 6 represents the entire thickness of the valve. On the aortic side one recognizes the scattered cocci adherent to, and included by phagocytosis in the endothelial cells, without much evident alteration of these latter elements. On the ventricular face of the valve, on the other hand, the endothelial cells contain many more bacteria. The endothelial cells themselves are swollen and are elevated irregularly by the accumulation of edema fluid in the subjacent stroma. In places there are small amounts of fibrin adherent to the free surface and these deposits sometimes contain recognizable erythrocytes. Such a small deposit is shown near the upper left corner of the drawing. Figure

13 represents a portion of the same aortic leaflet and shows the ventricular face at the site of the curve or kink, readily identifiable in the photomicrographs. Here phagocytosis of the cocci is also evident and at one place, at the right, a polynuclear leukocyte containing cocci is adhering to the endothelium. Of particular interest, however, is the lesion at the left of the figure, where the streptococci have already multiplied to form a dense bacterial colony with more or less advanced destruction of the endothelial cells. An irregular deposit of fibrin with incarcerated erythrocytes is attached to this altered surface and constitutes a minute, irregular projection which was grossly recognizable as an early vegetation. This is large enough to be seen in many serial sections.

In the sections of this heart there are also lesions in the myocardial vessels. Figure 9 represents a coronary arterial branch of a diameter of about 0.4 mm. and the interesting portion of the arterial wall is shown in Figure 11. At one place the endothelial cells of the intima have engulfed some of the streptococci and here there is a small adherent thrombus, evidently composed of fibrin, platelets and incorporated erythrocytes, and including easily recognizable streptococci. This lesion is evidently similar to those seen in the ascending aorta of this same animal. Figure 12 shows a thrombosed capillary in the ventricular myocardium. The thrombus extends along the capillary and contains polynuclear leukocytes and masses of bacteria toward the upper end in the picture. Toward the bottom of the figure the clot consists chiefly of fibrin and altered erythrocytes without visible cocci, suggesting an extension of the clot by the process of relatively aseptic thrombosis. This would appear to be a rather recent obstruction because of the slight changes in the adjacent myocardium. However, for decision in regard to mode of origin, even this vascular lesion is already too far advanced. Whether it started by endothelial phagocytosis of circulating streptococci or by the attachment of a polynuclear leukocyte already loaded with cocci remains uncertain but the latter mode of origin seems probable.

DISCUSSION

The significance of these observations in relation to the sequence of events in the development of the lesions of endocarditis requires little discussion because the evidence is in itself so clear. Obviously, an endocardial lesion might develop by extension of the inflammatory process from a thrombosed capillary near the endocardial surface, such as that shown in Figure 3 (rabbit 36). This might easily be accepted as the mode of origin of some mural vegetations. In our experimental

rabbits, however, such thrombosed capillaries are found after considerable search, whereas the endothelial cells of the mural endocardium may everywhere serve as phagocytes for cocci. It seems, therefore, that the great majority of the valvular and mural lesions as well as those of the aortic and arterial walls take origin from the intimal implantations of the circulating bacteria. Subsequently the endothelial cells may destroy the included cocci so that the lesion heals without residual damage, and this evidently takes place over considerable areas of the endocardium, as will be evident in the study of later stages of this disease. Failing this, the lesion may progress to cause more serious structural alteration and functional incapacity. This failure to heal evidently depends upon several factors: first, the virulence of the infecting bacteria; second, the general resistance of the host; third, other miscellaneous circumstances. Without entering too fully into these questions at the moment, we may here point out the contrast between the two surfaces of a heart valve. As may be seen in the drawings, it is the auricular surface of the mitral valve and the ventricular surface of the aortic valve which seem more favorable to the proliferation of the bacteria. This, we believe, is due to the greater physical trauma to which these surfaces are exposed. Each mitral leaflet comes into contact with its fellow at each systole of the ventricle so that the endothelial cells on the auricular surfaces over the area of this contact are closely applied to each other and they are peeled apart at each ventricular diastole. Similar contact under pressure affects the ventricular surfaces of the aortic leaflets. Physical relationships of pressure, speed of blood flow, stagnation of the blood and contact with other solid elements doubtless also play a part in the relative frequency of lesions in the ascending aorta, sinus of Valsalva, arteries, capillaries and veins, but the present experimental material does not warrant any extended discussion of these relationships.

SUMMARY

1. Following the intravenous injection of large amounts of washed bacteria as well as untreated cultures of *Streptococcus viridans* into rabbits, the bacteria are taken up extensively by phagocytosis by the endothelial cells of the endocardium and of the intima of the aorta and coronary arteries.
2. The bacteria also lodge in the myocardial capillaries either by direct endothelial phagocytosis of streptococci or by arrest of sluggish leukocytes containing the bacteria.
3. After phagocytosis many bacteria are evidently destroyed without production of recognizable persistent structural changes.

4. In some places, particularly on the heart valves, the included bacteria tend to survive, multiply, and initiate the precipitation of elements from the blood so as to give rise to the vegetations of endocarditis.

5. Local physical factors play some part in the progress of these local lesions.

This paper is a report of the results of joint effort. The experimental work on the animals was performed for the most part by Miss Spence. The microscopic sections were prepared by Miss Slavkin. The illustrations in color were drawn by Dr. MacNeal.

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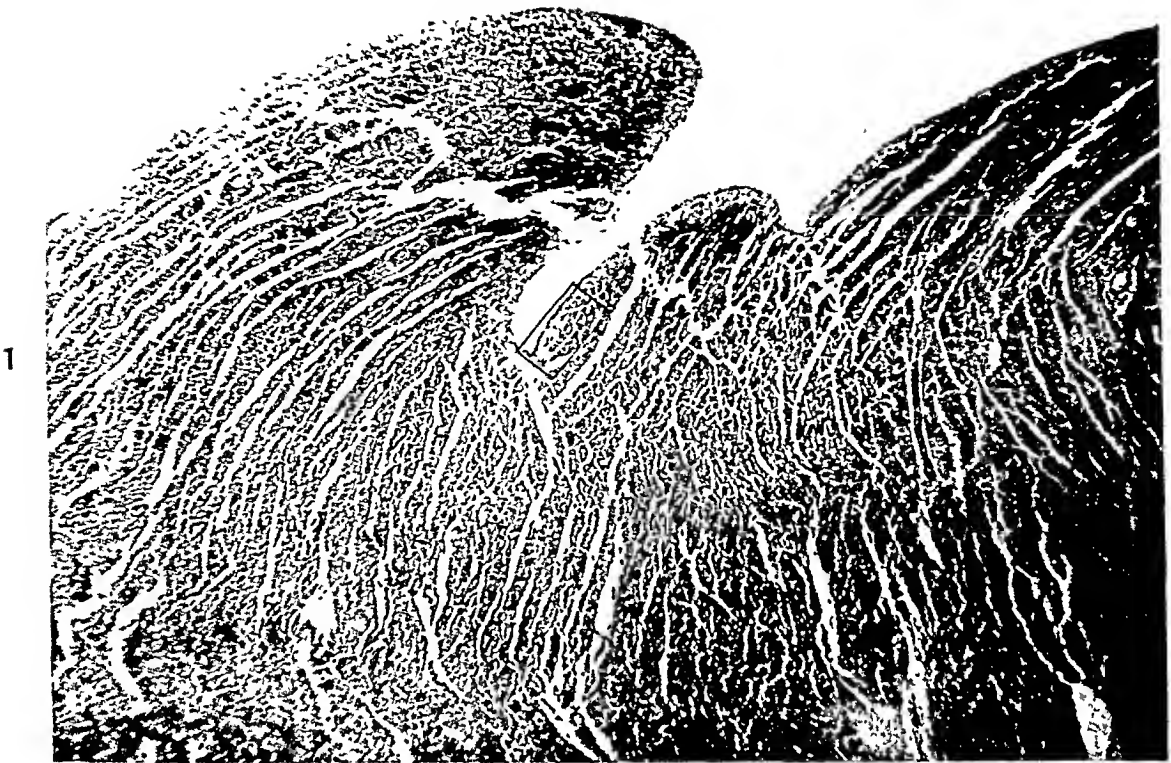
DESCRIPTION OF PLATES

The order and orientation of the illustrations have been arranged to meet the requirements of economy and artistic reproduction. By attention to the legends the reader may avoid confusion.

PLATE 87

FIG. 1. Rabbit 36. Section through the wall of the right ventricle. Photomicrograph at low magnification. Abnormalities might easily be overlooked in this picture. The portion outlined by the rectangle is shown at higher magnification in Figure 3.

FIG. 2. Rabbit 33. Section through the aortic valve including a valve leaflet, part of the ventricular wall and part of the wall of the ascending aorta, stained by the method of Brown and Brenn.² Photomicrograph at low magnification. One may recognize small areas of infection on the auricular face of the valve and on the intima of the aorta. The locations of the fields represented in Figures 5 and 8 are indicated by short heavy lines near the endothelium.



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PLATE 88

FIG. 3. Rabbit 36. Section through part of the wall of the right ventricle stained by the method of Brown and Brenn² and drawn by camera lucida, objective 60 X, N. A. 1.40, and ocular 4, at the magnification indicated by the included scale, which is standard for all colored drawings of this paper. The endocardial endothelial cells contain cocci and there are minute bits of clotted blood adherent to them in places. At about 0.1 mm. beneath the endocardium there is a thrombosed capillary containing many streptococci. Below the main drawing, this same capillary is shown in four successive serial sections of which the third in the series is the section represented in the larger drawing. It would appear that the thrombosis of the capillary has no local relation to the infection of the endocardial endothelium, which is quite general over the entire lining of the ventricle. The V is in the cavity of the right ventricle.

FIG. 4. Rabbit 34. Section through a mitral leaflet stained with hematoxylin and eosin and drawn by the same standard technic. The auricular face is roughened, and attached to it are bits of clot containing erythrocytes. The adjacent endothelial cells contain engulfed streptococci. The smooth endothelium on the ventricular face presents a sharp contrast. The V is in the cavity of the left ventricle.

FIG. 5. Rabbit 33. Drawing by camera lucida, objective 60 X, N. A. 1.40, and ocular 4, at the standard magnification indicated by previous scales. This drawing shows a portion of the ventricular wall with streptococci in some of the endocardial endothelial cells at the upper border of the figure. A small capillary lying just beneath the endocardium is cut longitudinally for some distance and appears to be normal. The myocardial cells are fragmented.

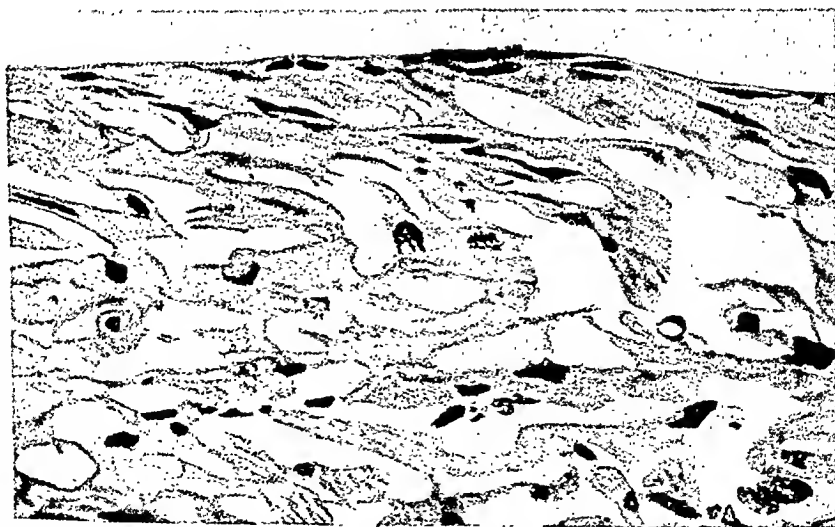
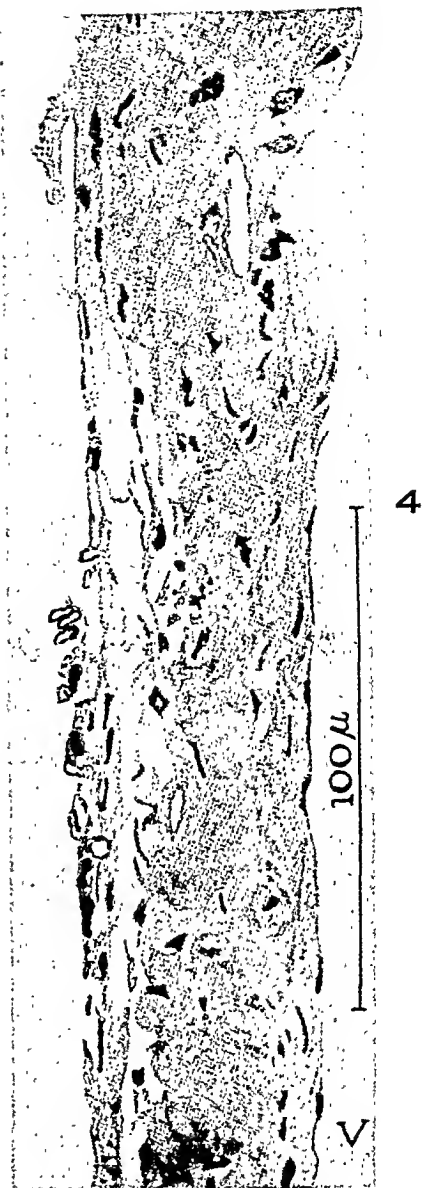
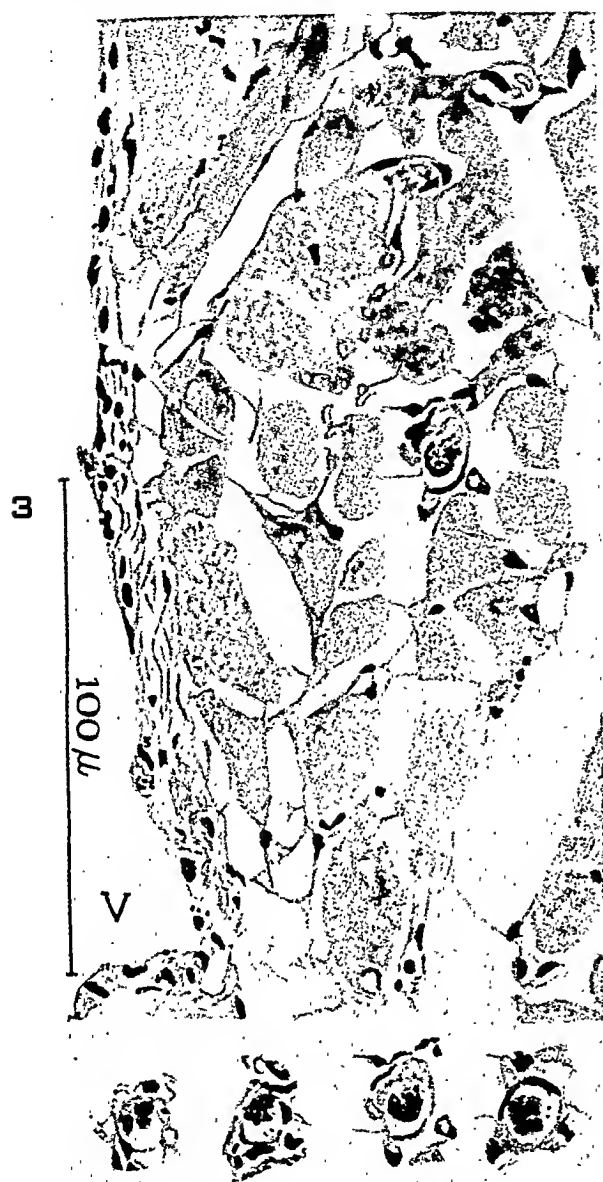
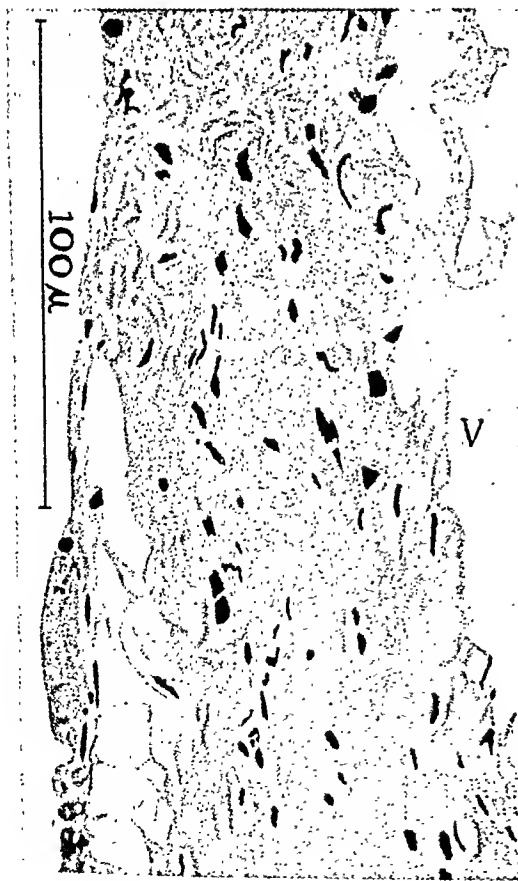
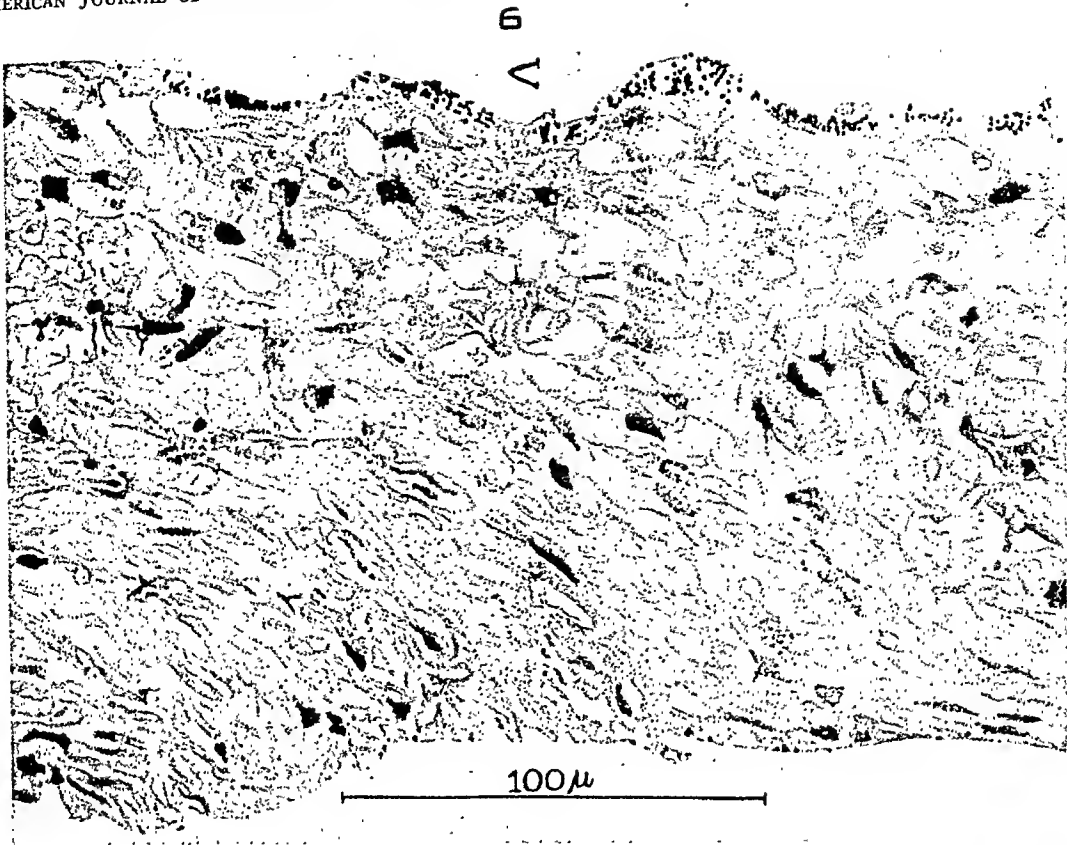


PLATE 89

- FIG. 6. Rabbit 33. Drawing by the standard technic of part of the aortic leaflet in the same section as that shown in Figure 10. The endothelial cells contain cocci which are more abundant on the ventricular face (V) of the leaflet, where there is also evidence of more anatomic alteration. The location of this field is indicated in Figure 10 (V).
- FIG. 7. Rabbit 34. Another section through a mitral leaflet stained by the method of Brown and Brenn² and drawn by the same standard technic. There are a few distinct bacteria in the endothelium on the auricular face of the leaflet and there is also an adherent clot nearly 0.1 mm. in extent, with erythrocytes and bacteria in it. The V is in the cavity of the left ventricle.
- FIG. 8. Rabbit 33. Drawing by camera lucida, objective 60 X, N. A. 1.40, and ocular 4, at the standard magnification for all colored drawings. This shows a small part of the wall of the ascending aorta. Some of the endothelial cells of the intima contain streptococci. In the upper half of the drawing the proliferation of the bacteria is associated with local necrosis.



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PLATE 90

FIG. 9. Rabbit 33. Photomicrograph of a coronary artery showing at one place on the intima (lower right) a small vegetation. The artery is about 0.4 mm. in diameter.

FIG. 10. Rabbit 33. Same section as that shown in Figure 2 but photographed at a higher magnification to show more detail of the valve leaflet. The small vegetation on the ventricular face of the cusp may be recognized, as well as the collections of bacteria in the endothelium.



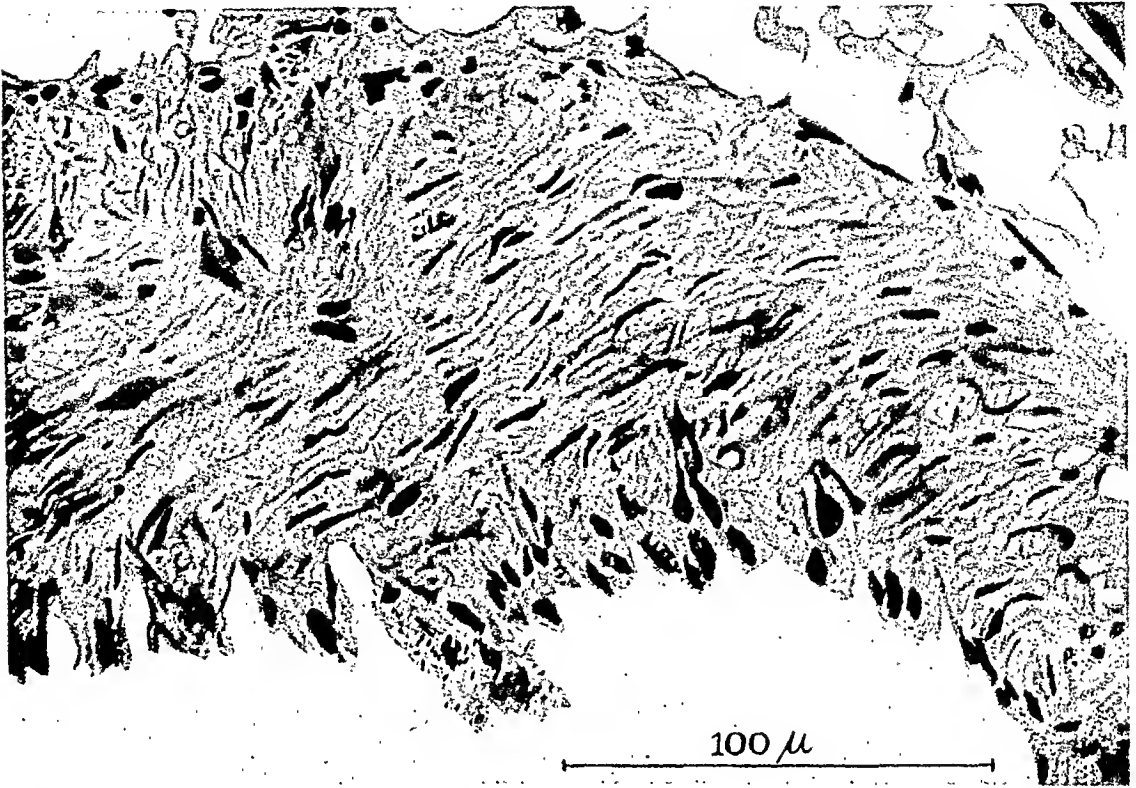
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PLATE 91

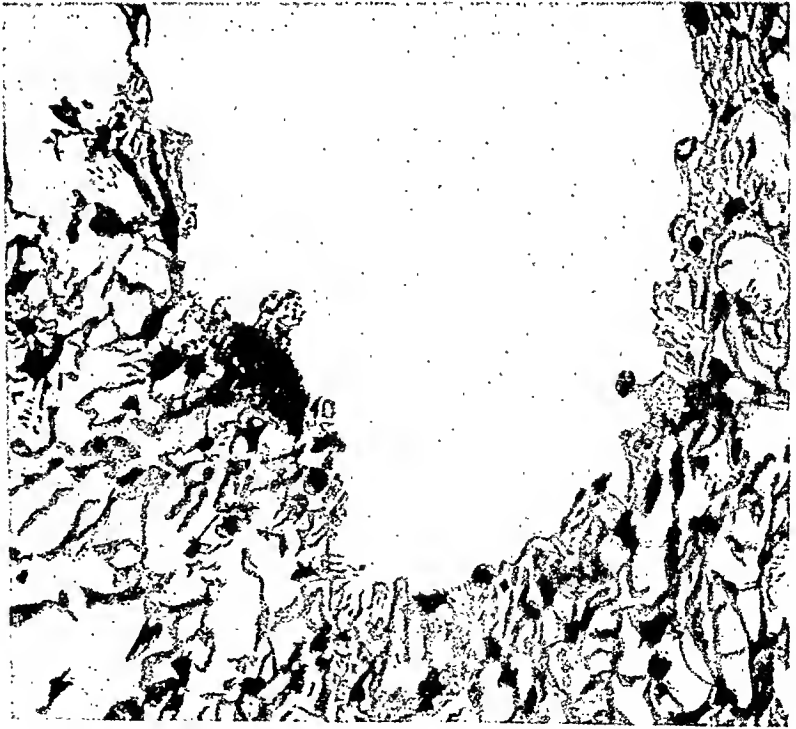
- FIG. 11. Rabbit 33. Drawing by camera lucida by the standard technic. Here there is shown the small vegetation on the intima of the coronary artery seen in Figure 9. The intimal endothelium contains included cocci and clinging to it there is a deposit of fibrin in which there are erythrocytes and bacteria. The other arterial coats appear to be unaltered.
- FIG. 12. Rabbit 33. Drawing by the standard technic. There is shown a thrombosed capillary in the ventricular myocardium. The thrombus is purulent, with abundant streptococci in the upper part of the longitudinal section. The lower part consists chiefly of fibrin and erythrocytes (aseptic extension of the thrombus). There is little reaction in the adjacent myocardium.
- FIG. 13. Rabbit 33. Drawing by the standard technic of part of the aortic leaflet in the same section as that shown in Figure 6. Here the minute vegetation is included at the left. It has in it erythrocytes, fibrin and bacteria. At the right a polynuclear leukocyte with three included cocci adheres to an endothelial cell. The location of this field is indicated in Figure 10, in which the small vegetation serves as a landmark.

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CHARACTERISTICS OF A LIPOSARCOMA GROWN IN VITRO *

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The recent review of adipose tissue by Wells¹ emphasized the facts that fat is a definite tissue often organoid in character and that its cells are probably not just modified fibroblasts but are derived from units which are segregated from undifferentiated mesoderm during embryonal life and persist throughout postnatal life as specialized lipoblasts.

It would seem that embryologically lipoblasts are found in two different forms: in one they are contained in tissue resembling vascular mesenchyme with widely spaced stellate or spindle-shaped cells surrounded by mucoid intercellular substance and only gradually become rounded as fine droplets of lipid appear within the cytoplasm; in the other the cells are rounded from the beginning and grouped in gland-like lobules of a moruloid or mulberry appearance. In this form there is no intercellular mucin but the fat forms first as intracellular droplets. Both tissues are highly vascular.

It is particularly important to know about the existence of these two embryological forms when studying the malignant lipoblastic tumors because both are reproduced in the growth of liposarcomas.

The liposarcomas which reproduce the aspect of mesenchymal tissue are generally myxomatous and slimy but occasionally are composed only of fibrosarcoma-like tissue mixed with lipoblasts. Both variants usually have an admixture of adult fat cells and fat-laden phagocytes. In the second form of liposarcoma only rounded or polygonal lipoblasts of varying sizes and adult fat cells are present without any myxomatous or fibroblastic tissue. In the material at our disposal, consisting of 24 cases, there were 19 of the myxoid tumors and 2 round-cell forms. In 3 other cases the tumors were made up of solid masses of rounded cells in some areas and of myxoid tissue in others, so that they were composites of the two different forms of liposarcoma. This suggests that the lipoblast is probably a single type cell which can produce both myxoid and adenoid embryonal adipose tissue.

Liposarcomas in our experience were twice as common in males as in females and they occurred in patients with an age variation of from 28 to 82 years, with an average for the group of 56.5 years. Although

* Received for publication, January 11, 1943.

they may occur in a wide variety of regions, the great majority have developed either retroperitoneally, with a preference for the perirenal area, or in the region of the thigh, including the popliteal space, the groin and buttock. In general they tend to grow slowly to a very large size, and infiltrate surrounding tissues. Because of this they are difficult to eradicate by operation. Metastases, both in our own group and in reported cases, are known to have occurred in 20 per cent. In a small number there have been multiple tumors.

In the present communication we wish to present the cultural characteristics of a liposarcoma, since so far as we are aware such observations have not yet been recorded. The tumor developed in the subcutaneous tissues of the upper radial aspect of the right forearm of an American negro laborer, 64 years old (Fig. 1). In $8\frac{1}{2}$ months it reached a size of 20 by 8 by 6 cm. and was then excised, the wound being treated with x-ray irradiation. Two courses were given during the next 5 months totaling 46 treatments through four 8 by 10 cm. fields. The factors were 200 kv.; 25 ma.; target-skin distance, 50 cm.; filters, 1 mm. Cu. and 1.25 mm. Al. The total dosage was 12,050 r.* Two years after excision a metastatic lump appeared in the subcutaneous tissue over the upper inner aspect of the right biceps brachii muscle, surrounding the cephalic vein and extending deeply between the deltoid and biceps muscles as far as the periosteum of the humerus (Fig. 1). It was excised and measured 4 by 4 by 3.5 cm. It was from this specimen that the explants were made. This area was also treated postoperatively by roentgen therapy, using three 10 by 15 cm. fields with the same factors as before and reaching a total dosage of 4,850 r.

Two months after the second operation a recurrent nodule appeared in the scar of the first operation in the forearm. This was treated with radium needles and a dosage of 1425 mg. hours was given. When the patient was last seen over 2 years later, 57 months after the first operation, there was no clinical evidence of local disease and no x-ray evidence of pulmonary metastases.

Both gross specimens were nodular, with mottled yellowish white cut surfaces glistening with sticky mucoid material. The original tumor when cut allowed the escape of a good deal of gelatinous material, brownish red fluid, and soft reddish yellow neoplastic tissue. The metastatic nodule was solid throughout.

Microscopically the original tumor showed a framework of fibrous tissue bearing blood vessels which supported an extremely loose-textured tissue containing widely spaced cells of a most diverse appearance (Fig. 3). The majority were relatively large and irregularly stellate

* Wherever "r." is used, the value indicates roentgens measured in air.

but multinucleated syncytial masses were frequent and elongated ribbon forms not uncommon. The nuclei were often hyperchromatic with prominent nucleoli and there were occasional bizarre mitoses. The cytoplasm often contained lipid droplets. There were a few signet-ring cells. The intercellular substance had a few delicate reticulin fibers and a great many spaces which contained the mucoid material. This could not be stained with mucicarmine. There were occasional groups of adult fat cells.

The metastatic nodule from which the explants were made retained the characteristics of the original tumor but suggested much more active growth. The tumor cells were proportionally much greater in number and the intercellular substance correspondingly less. Mitosis was at the rate of one per high power field. Intracellular lipid droplets (Fig. 3) were frequent in some areas and absent in others. Lipoid-filled signet-ring phagocytes were relatively more frequent than in the primary growth and again there was no mucicarminophilic intercellular substance (Fig. 3). The tumor was now definitely invasive and not sharply circumscribed.

TISSUE CULTURE

Methods

Cultures were made from the metastatic lump removed from the upper arm 2 years after excision of the first tumor from the forearm. The Maximow double-coverslip, lying-drop method² was used, and the tissue was explanted in a medium consisting of 4 parts chicken plasma, 4 parts human placental serum, 1 part extract of adult rat spleen, and 3 parts buffered saline (according to Gey and Gey,³ with slight modification). The cultures were washed and refed three times a week, being transferred when necessary; for this rapidly dividing tissue, passage would average once in 8 to 10 days. They were maintained for 51 days.

Growth Characteristics

The cultures grew profusely and regularly after a short lag-period of 18 to 24 hours. The outwandering of a multitude of leukocytes, monocytes and macrophages preceded growth of tissue. The tumor growth contained a few signet-ring cells of the adipose type, but these in general were inert from the standpoint of both reproduction and locomotion, and were probably drawn out passively from the explant by the contraction of the clot or by the advancing ranks of fusiform cells which formed the main body of the outgrowth. The content of the signet-ring vacuoles, however, was not fat, as ascertained by scharlach

R staining; neither could it be stained by mucicarmine nor by the metachromic dye—toluidine blue. Possibly this condition is related to the serous atrophy of fat described by Wells.¹ Often these cells progressively resolved their contents and became ameboid and spindle-shaped (Fig. 2).

The characteristic growth from the tumor (and the first tissue cells to emerge) consisted of rather fat, bright, spindle-shaped, discrete cells, with a round or oval vesicular nucleus and one or two prominent nucleoli (Fig. 5). There was considerable variation in size of both cell and nucleus. Binuclearity was common, and in the later stages of cultivation some exceedingly bizarre, lobated and fragmented nuclei made their appearance (Fig. 4).

Although the tumor was grossly slimy, we obtained no clear results in the cultures from methods designed to stain mucin. With scharlach R, however, we were able to stain many fine granules in the cells; but large vacuoles (Fig. 6) which we expected to react as fat, did not. These cells did not liquefy their plasma medium to any extent. The tumor cells did not show cement borders which blacken with silver nitrate, as do endothelial and mesothelial cells, and they never formed membranes nor the interlacing fretwork that is characteristic of fibroblastic growths. But, after about 2 weeks *in vitro*, fibroblasts and endothelium made their appearance in some cultures, especially from the necrotic edge of divided cultures. Both the branching form and the nuclear pattern (two to seven small nucleoli) of the fibroblast served to distinguish it from the lipoblast.

The cells of this tumor reacted sharply to mechanical disturbance, by contracting and remaining inert for varying periods. After transferal from one slide to another it might be as long as 3 or 4 days before cell division became frequent again. The initial "growth" of the tumor cultures appears to have been an outward migration of tumor cells, among which there were extremely few mitoses. But after this initial phase was overcome, there were as many as 75 mitoses in a culture at one time. Estimating the time required for a division to be about 1 hour, and assuming the rate of division to be constant for 24 hours, there would be 1800 mitoses during a day at the peak of activity for one of the most active cultures.

Fifteen days after the original cultures were made, a second set was prepared from the remainder of the tumor specimen, which had been kept in the refrigerator at 2° to 4°C. during that time. These cultures showed a longer lag-period—3 days or more—and produced a sparse growth, the nature of which remains undetermined.

When the original cultures were 12 days old, it was decided to use

some of them for preliminary experiments in x-ray irradiation, with the co-operation of Dr. Maurice Lenz, who was interested in the radiosensitivity of these tumors. Since we had only nine cultures whose rate of growth had been constant enough to warrant experimental use, the results can be regarded only as suggestive. These were rated from one plus to three plus on the basis of growth rate, and paired with untreated controls of parallel growth energy. Two experimental cultures, paired with two controls, were given 550 r. = 5 min., and three experimental cultures, paired with two controls, were given 1100 r. = 10 min. The factors were: 81 kv. constant potential machine, 4 ma., 25 cm. target distance, no filter, 110 r. per minute. Erythema dose = 275 r.

Irradiated cultures and controls were then kept for 1 month and compared in respect to growth (*i.e.*, number of mitoses, nutrition and cell morphology). The growth for the first 2 days was nil, among the experimental cultures. At the end of this time those which had received the smaller dosage began to divide. On the fifth day, those with the greater dosage began to divide, but the rate always lagged behind those with the light dosage, one of which was even with its paired control. Among the treated cultures, those with the highest growth rate suffered most severely. The light dosage did not affect the nutrition of the cultures significantly, but the heavy dosage produced a profound effect.

So far as morphology is concerned, there were bizarre forms appearing here and there in all of the cultures as well as in the sections of the tumor, but in the irradiated cultures the proportionate number of these forms was greatly increased. It would appear that the viable, reproductive cells were the most affected by the radiation, in some cases leaving only these stranded, bizarre cells which, from the reproductive standpoint, were already out of the running.

DISCUSSION

In the tissue cultures of this liposarcoma the actively growing lipoblasts can be distinguished readily from common fibroblasts on grounds of nuclear as well as of cytoplasmic properties, and of general growth pattern. This is usually true, according to Hausberger,⁴ of young fat cells undergoing normal development, before they have yet begun to store fat. But although a general similarity has often been noted between embryonal cells and rapidly multiplying tumor cells, the comparison should not be pushed too far, and conclusions as to normal cell origin cannot properly be drawn solely from a study of malignant cells such as these. Still it seems worth while to state that these malignant lipoblasts appear to be a distinct type of cell, and that they are rather similar to the stem cells which appear in cultures of indifferent mesen-

chyme. The typical viable, reproducing, spindle-shaped tumor cell has a number of points in common with Chlopin's ⁵ "desmoblast" of indifferent mesenchyme.

It may be of some value, further, to make reference to the work of Burkhardt ⁶ in cultivating adipose tissue from the bone marrow of adult guinea-pigs. He showed that the exaggerated capacity developed by adipose cells for fat storage is not coincident with the loss of other mesenchymal potencies. Fat cells appeared in his cultures sometimes as multinucleate giant cells which quickly degenerated, sometimes as fibroblast-like cells, more often as ameboid, wandering cells. They were capable of locomotion and phagocytosis, and even of mitotic division while containing rather large fat vacuoles. This great variability in form which they exhibited he regarded as exemplifying "the morphological richness which characterizes vascular mesenchyme" from which he believes adipose tissue to be an offshoot.

It is interesting that in both sections and cultures of the liposarcoma which we have described, the gamut of cells discussed by Burkhardt ⁶ is run. It is our observation also that the bizarre, multinucleate cells are relatively nonviable, and are on the verge of degeneration. Lipoblasts distended with vacuoles may resolve these partially and become ameboid (Fig. 2). The tumor cells may also develop the cytoplasmic (though not the nuclear) aspects of fibroblasts, while containing large vacuoles (Fig. 6). In our cultures these large vacuoles have not contained fat, but may have illustrated the capacity, referred to by Wells,¹ of depleted fat cells to take up water.

SUMMARY

The two histological types of liposarcoma are discussed briefly, and the clinical and histological aspects of one case are recounted.

Portions of a metastatic nodule from this case have been cultivated *in vitro*, and the growth characteristics are described. These malignant lipoblasts appear to be a distinct type of cell, though highly variable in form.

Some of the tissue cultures of this tumor have been exposed to x-ray irradiation, and their subsequent course is summarized.

The authors' thanks are due to Dr. Cloyce F. Bradley, who assisted in the preparation of the tissue cultures.

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[Illustrations follow]

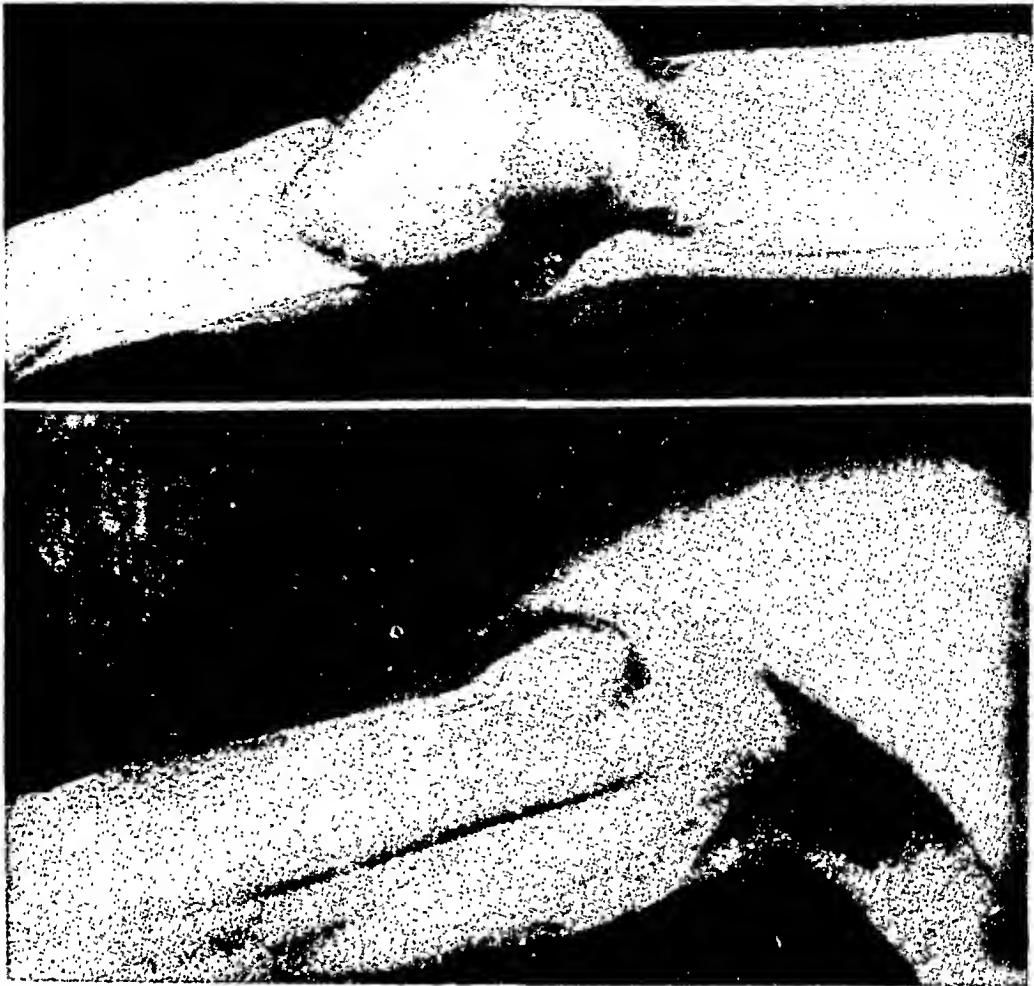
DESCRIPTION OF PLATES

PLATE 92

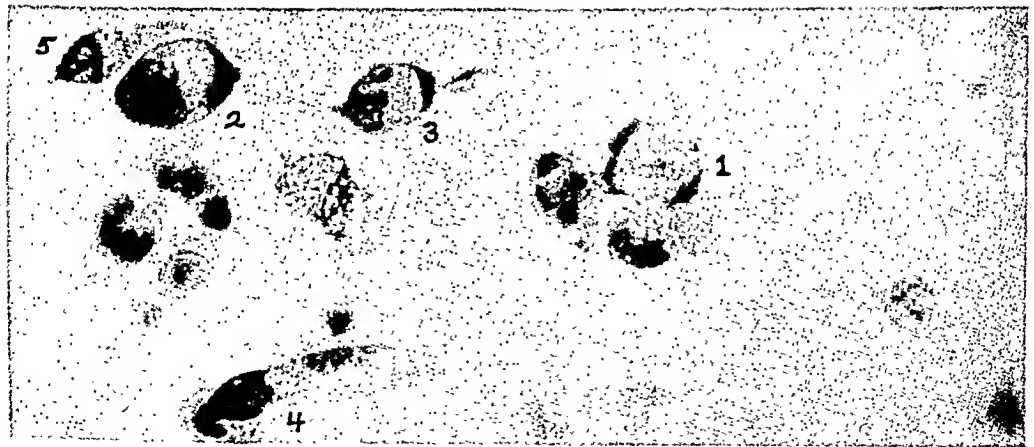
FIG. 1. Liposarcoma of the forearm. Above is the primary tumor and below the metastasis in the upper arm.

FIG. 2. Lipoblasts at the periphery of outgrowth from 4-day culture. There are signet-ring cells in various stages of elongation and vacuole resorption: 1, 2, 3, 4, 5. Formaldehyde fixation, toluidine blue stain. $\times 680$.

1



2



Murray and Stout

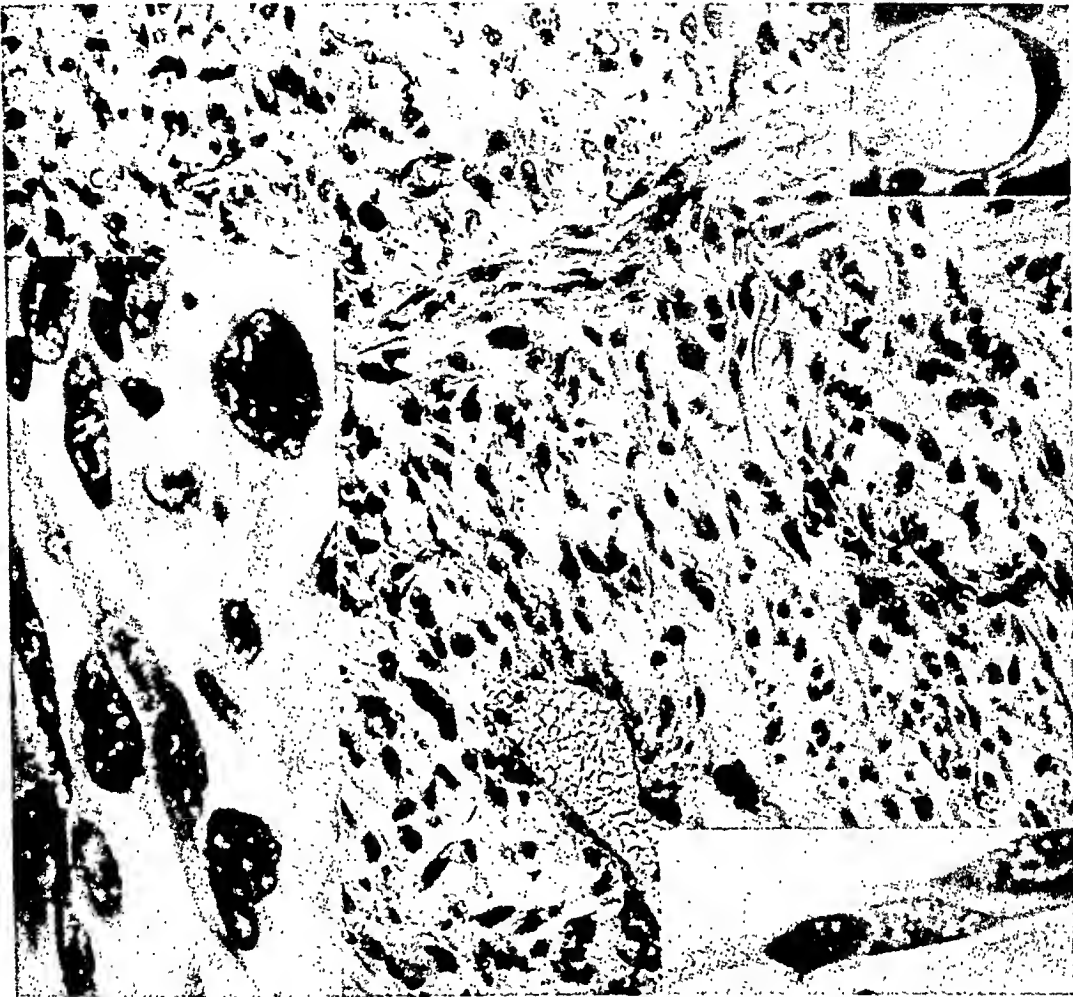
Liposarcoma Grown *in vitro*

PLATE 93

FIG. 3. The low-power photomicrograph shows the characteristics of the original tumor in the arm. The insets show cells of the metastasis from which cultures were made. Bizarre elongated cells (lower left); signet-ring cell (upper right); lipoblast with vacuoles (lower right). $\times 630$.

FIG. 4. Tumor cells from 42-day culture, irradiated 550 r. = 5 min. Note disparity in cell size, vacuoles, and lobated nucleus in large cell. Helly's fixative, mucicarmine stain. $\times 380$.

3



4



Murray and Stout

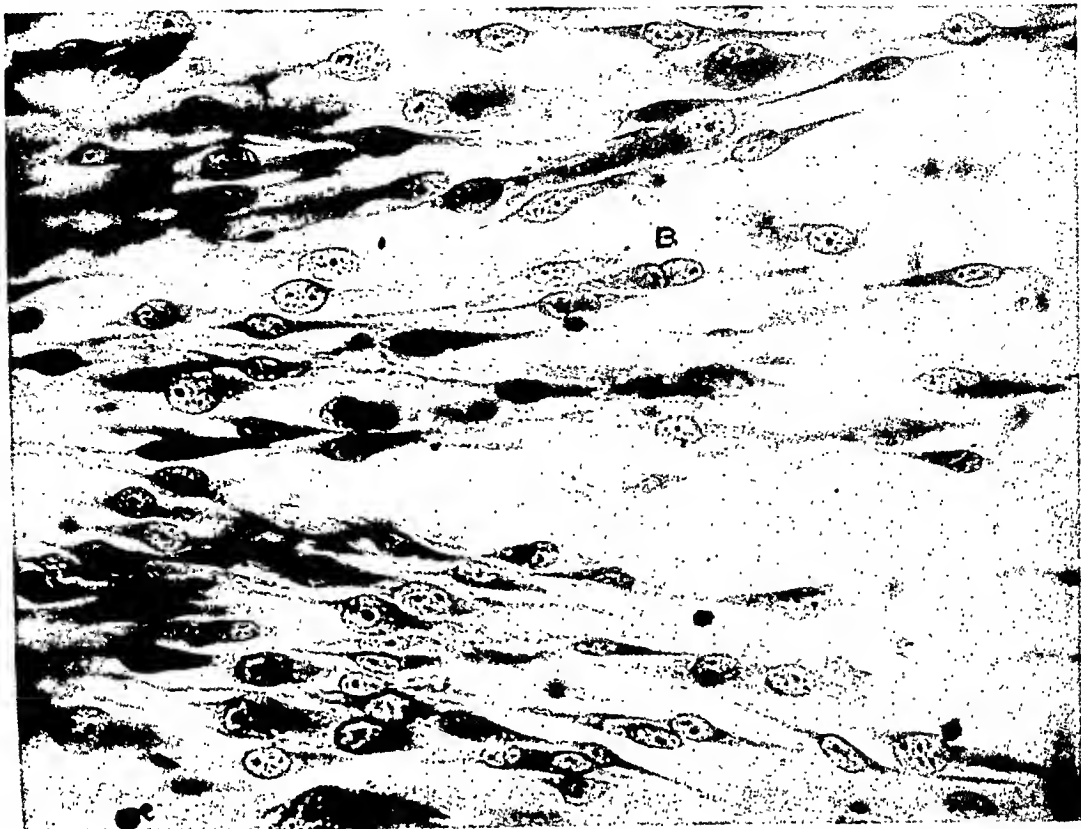
Liposarcoma Grown *in vitro*

PLATE 94

FIG. 5. Typical outgrowth of viable, reproductive cells after 7 days *in vitro*. Note spindle shape, 1 or 2 nucleoli, and lack of uniformity in size among the cells. B = binucleate cell. Zenker's fixative, fuchsin-ponceau-aniline-blue stain. $\times 380$.

FIG. 6. Large watery vacuoles in branching tumor cells of 42-day experimental culture (550 r. = 5 min.). Helly's fixative, mucicarmine stain. $\times 380$.

5



6



Murray and Stout

Liposarcoma Grown *in vitro*

EPITHELIAL CYSTS AND CYSTIC TUMORS OF THE SKIN *

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Cysts of the skin form a heterogeneous group which is difficult to subdivide on either an etiologic or histologic basis, as a cyst often carries no earmark indicating its origin. A reinterpretation of the abundant literature on cutaneous cysts would be impractical and of doubtful value as well, because so much of it is vague and uncritical. This review is based largely on our observations of 566 epithelial cysts of the skin examined in this laboratory over a 20-year period. Recent reports of large numbers of cysts by Caylor,³ who reported on 236 "cutaneous cysts," Bishop,¹ reporting 119 "sebaceous cysts," and Stone and Abbey,¹⁰ also reporting on 363 "sebaceous cysts," have been especially helpful.

Cysts of the skin may be put into one or another of three main groups according to their origin: (a) aberrant squamous epithelium, either congenital or traumatic in origin; (b) overactivity of glands, resulting from general physiologic forces, external conditions, or from some unknown factor; (c) degenerative changes of skin appendages or of benign or malignant epithelial tumors.

A. CYSTS

1. Epidermal Cysts

The commonest type of epithelial cyst of the skin is the epidermal cyst which is lined entirely by stratified squamous epithelium usually without skin appendages. All but 10 of our 566 cysts fall in this group. These cysts may arise from hair follicles, sebaceous ducts and from the epidermis, and it is usually impossible to determine the parent structure. Many different terms have been applied to them, generally depending on the probable structure of origin, as: sebaceous, retention, follicular cysts, atheroma, pseudo-atheroma, steatoma, milia, seboid-epidermal cysts, sebaceous tumors, oil cysts, squamous epithelial cysts, pilosebaceous cysts, sebaceous cysto-adenomas, keratomas, simple dermoids, and epidermal inclusions.^{2, 5}

The cysts which are clearly derived from one or another of the cutaneous organs will be discussed separately.

The simple epidermal cyst lies in the corium, rarely in the subcutaneous tissue. Those cysts occurring along embryonic lines of

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closure and situated deep in the corium have been assumed to be congenital inclusions. Some of the cysts are joined to the surface by epidermal cords, suggesting that for some reason abnormal downward prolongation of the epidermis had resulted in a cyst. But the majority of these cysts are not connected with any cutaneous structure. Injury to the skin may produce epidermal cysts by misplacement of epithelium or stimulation of epithelial proliferation.

These cysts contain compact, fine flakes of epithelial cells. The contents may disintegrate into amorphous debris with fat and cholesterol or they may become hard, keratinized tumors and often calcify. A similar material found in sebaceous cysts is more fatty and is distinguished by the characteristic rancid odor. Internal papillae and ridges have been described as features of cysts formed from epidermal inclusions.^{12, 13} Absence of any connection with the skin surface distinguishes them from follicular or sebaceous cysts.

The majority of cysts that are removed show rupture of the capsule with inflammatory reaction. A foreign body giant cell reaction and mononuclear cell infiltration are elicited by the contents of the cyst which are forced into the fibrous tissue of the corium. This reaction may resemble a lipophagic granuloma^{6, 7} (Fig. 1). The portions of cyst wall which are isolated often proliferate actively and may simulate a malignant, infiltrating growth. A few cysts remain stationary; others undergo spontaneous involution, with fibrosis, calcification, and occasionally ossification. Eleven of our 566 epidermal cysts were calcified; and one cyst was ossified. Malignant change is probably extremely rare.^{4, 8, 9-11} We have encountered carcinomas arising from the walls of four epidermal cysts (Fig. 2).

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2. Epidermal Cysts of Traumatic Origin

Trauma has long been considered an important factor in the formation of epidermal cysts¹ which have been variously diagnosed as traumatic, post-traumatic,¹³ implantation epidermal or implantation dermoid cysts.⁵ This assumption has been corroborated experimentally. Kaufmann¹² found that cocks' combs buried in subcutaneous tissue formed cysts lined by stratified squamous epithelium. It has also been thought (erroneously) that cysts may develop from epithelization around foreign material such as a magnesium disk or camphor oil after it has been absorbed.^{15, 17}

Trauma may produce cysts in three ways: (a) by displacement of epithelium; (b) by altering the structure of cutaneous organs, such as hair and glands; (c) by stimulation of epithelial growth. Cysts sometimes follow continued irritation, through stimulation of growth of epithelial structures in their natural location.¹⁰ Kummer and Christiani¹⁴ reported epithelization and cyst formation at the end of an embedded nail. This epithelization is said to derive from epithelium of skin appendages, particularly the ducts of coil glands.¹³ Franke⁹ suggested that embryonic rests pinched off from the epithelial pegs might become cystic as a result of trauma. Although trauma plus irritation of normal cutaneous epithelial structures or of dormant embryonal rests may be the primary cause of some cysts of the skin, the evidence is not always enough to establish the cause. Such a diagnosis has often been made on insufficient grounds.⁷

Implantation cysts form one variety of the traumatic cysts. In this group may be included the cysts that develop in postoperative scars,^{2, 20, 26, 28} and probably some of the cysts said to be common on the fingers of laborers.¹⁰ Tooker²³ reported a post-traumatic, presumably implantation cyst in the posterior chamber of the eye and Couch⁴ reported one beneath the periosteum of the skull. Wien and Caro²⁵ suggested that subcutaneous implantation of epithelium would be more apt to occur from injury with a blunt or tearing instrument.

But Unna²⁴ cited a case of epidermal cyst which was said to result from a needle prick, and he expressed the opinion that the implant is more apt to survive if spherical, since irregular implants stimulate granulation tissue and may be absorbed.

In most instances no distinction can be made between cysts resulting from implantation of epithelium and those from epithelial proliferation *in situ*, and in any case such a distinction has no practical importance.

There is no very clear information as to the incidence of traumatic epidermal cysts, although several single cases as well as groups of cases have been reported.^{3, 13, 16, 18, 22, 25} Wörz²⁷ reported a series of 55 cases in which 44 per cent of the cysts were said to be related to injury.

The latent period may be anywhere from 25 days to 25 years.¹⁸ The rate of growth is also variable and both infection and calcification occur. Cogswell and Goodale³ reported one cyst that eroded the terminal phalanx of the finger. Franke⁸ reported malignant proliferation in what may have been a true traumatic epidermal cyst of the base of the thumb. Some epidermoid carcinomas developing after injury have been attributed to malignant change of misplaced epithelium, but without actual proof.^{6, 11} Certain adamantinomas of the tibia have been said to originate the same way.²¹

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3. Sebaceous Cysts

The term sebaceous cyst has been made so inclusive that it has lost its essential meaning. Virchow¹⁶ considered all epithelium-lined cysts of the skin containing semisolid or fluid contents to be formed from sebaceous glands. This concept has been generally accepted with very little critical consideration. Several rather loose terms have accumulated for the main class of sebaceous cysts or certain histologic variations thereof: ¹ "calcinoses," ¹⁷ "sebo-epidermal cysts," ¹¹ "steatoma," ¹² "atheroma." ¹⁰ Stelwagon and Gaskill¹² derived sebaceous cysts from embryonic misplacements. Franke⁸ and Török¹⁴ were the first to differentiate epidermal cysts, "atheromas," and sebaceous cysts; but others, such as Sulzberger and Wolf,¹³ while recognizing the distinction, did not apply it clearly.

The tendency to inflammation and degeneration shown by all types of cysts makes it frequently impossible to tell one from the other.

Unna¹⁵ thought that cysts of the sebaceous gland proper had not been shown to exist, and that so-called "sebaceous cysts" are really epidermal cysts and may be formed by a downward keratinization of the pilosebaceous follicle and later distention to form cysts. Broders and Wilson³ also concluded that most sebaceous cysts are "keratomas," that is, epidermal cysts filled with laminated keratin.

The frequency of malignant change in sebaceous cysts has been placed as high as 9.2 per cent,² but Caylor's⁴ more conservative figure, 3.4 per cent, is more in accord with the general impression.^{7, 9}

We have accepted the definition, long in use, of sebaceous cysts as retention cysts of sebaceous glands.⁶ Secreting sebaceous gland cells must form an integral part of the lining. We have identified only 3 such cysts among 566 cutaneous epithelial cysts, comparable to Collins'⁵ experience (Fig. 2). A helpful gross diagnostic point is the homogeneous, yellow, buttery consistency of the contents of sebaceous cysts as contrasted with the waxy, flaky, white material characteristic of epidermal cysts. Infection gives a darker color and a rancid odor to the contents of sebaceous cysts.

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4. Cystic Dilatation of Sweat Glands

The only true cyst of the sweat gland involves the deep part of the duct and is usually called hydrocystoma.⁹ Other cysts are less clearly neoplastic, as, for example, the transient cystic dilatation of the terminal duct seen in sudamina⁸ or prickly heat, and the dilatation of coils which occurs in a variety of conditions.

Cystic dilatation of sweat glands was described first as hypertrophy by Verneuil,¹² later as dysidrosis by Jackson,⁴ and then as hydrocystoma.^{1, 3, 5, 6, 9} Little has been added to the literature since these early reports. There have been a few recent reports.^{2, 7, 13} We distinguish this lesion from syringoma.

The cysts present as pinhead to pea-sized, shiny, translucent, somewhat deep and persistent vesicles on the face, especially the forehead, eyelids, and cheeks. Women in late middle life are said to be most often affected. A peripheral hyperemia is sometimes present about the large cysts. If unmolested, they may in time disappear as a result of excretion or absorption of the fluid, but occasionally they persist unchanged.

Relatively little is known of their natural history. They have been considered an anatomicophysilogic aberration, rather than a neoplasm.¹⁰ An excessive secretory activity is more or less borne out by the histologic structure and the multiplicity of lesions. In certain instances they seem to be associated with exposure to heat and moisture, either climatic or occupational. The importance of nervous influences is in great part hypothetical.^{3, 6}

The cysts may be large enough to cause some distortion of the adjacent corium but this is not usual. The lining is the flattened distorted epithelium of ducts, which apparently keratinizes in some instances.^{2, 7} Sutton and Sutton¹¹ described a sensitivity to radiation.

The only cyst of sweat gland which we have in our group of skin tumors had been excised intact and measured 0.8 cm. It was filled with glairy fluid and the inner surface was white, smooth and glistening. The epithelial lining, though partly exfoliated, was in other parts well preserved and consisted of tall columnar cells, the nucleus at the base and the clear cytoplasm toward the lumen, and having a basal layer of

flattened cells. Other partially cystic sweat glands were adjacent to this larger cyst. In the surrounding corium there was a slight inflammatory reaction, edema and hemorrhage. The overlying epidermis was unchanged except for being somewhat thinned.

While cysts of Moll's glands have been described, none was encountered by us.

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5. Dermoid Cysts

True cutaneous dermoid cysts are of necessity congenital and occur in relation to embryonal fissures and clefts. For their diagnosis the occurrence of epidermal epithelium and all skin appendages is requisite. In addition to these, various tissues of mesenchymal origin may be present. Dermoids of the skin are restricted to the corium and must be kept distinct from branchial cysts. At times their differentiation from traumatic epithelial cysts or from teratomas may be difficult unless the above criteria are followed. Whether there is any essential difference other than location between dermoid cysts of the skin and some of the simpler teratomas is questionable.

The contents of the cutaneous dermoid resemble the material within the more familiar ovarian dermoid cysts. Hairs are embedded in a pale yellow, greasy, pultaceous mass, which often has a faintly rancid odor. Usually these cysts do not reach a size over a few centimeters in diam-

eter, and growth is slow or negligible. Sometimes one or another element may be atrophic, hyperplastic, or rarely show malignant change.² Gans³ mentioned that calcification of the contents and subsequent ossification may occur.

True dermoids of the skin are rare.^{1, 4} The majority of those reported near the skin surface are either from branchial clefts or from the lines of closure of the nasal bones or skull.⁵ One, containing teeth, was reported as occurring in the external auditory canal.⁵

In our material we have encountered only two cutaneous dermoid cysts; each arising above the outer canthus (Fig. 3).

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6. Multiple Follicular Cysts

Cysts arising from the pilosebaceous apparatus were first reported by Bosellini² and Pringle.¹⁵

Abnormally active keratinization is usually considered the primary change which may give rise to cysts in the following manner: (1) gradual distention of a follicle from accumulation of detritus of dead keratinized cells; (2) blocking of the sebaceous duct; (3) accumulation of sebaceous secretion.^{13, 14} The sebaceous gland probably plays only an accidental or passive part, although it may add to the contents or make up part of the wall. In the older cysts, the sebaceous gland is often atrophic, and sometimes it is absent. It is obvious that the distinction from a sebaceous cyst might not always be possible, and there may be confusion with dermoid cysts.¹⁶

The cause of the hyperkeratinization is not known. Weidman²² has frequently found these cysts on tuberculous patients at autopsy and has suggested that avitaminosis may be a factor in their development. There is a general belief that the condition is hereditary.^{7, 17} Klausner¹⁰ and Sachs²⁰ reported the lesion in three successive generations, and Sachs reported one family in which ten members were affected. Weber²¹ believed that the condition occurs in two forms: one which is

mild and localized and not hereditary, and the other, which is more generalized and severe and a mendelian dominant characteristic.⁵

These cysts have certain clinical characteristics which seem to separate them from the main group of epidermal cysts. Multiplicity is the rule, several hundred sometimes being present,⁸ but solitary lesions have been described.^{6, 15} They are most common where the pilo-sebaceous apparatus is well developed and first appear on trunk, scrotum and axilla. Many of the patients have a long history of seborrhea or acne.^{9, 11, 12} The cysts are often present at an early age but may attract attention first in middle life.^{3, 4, 19}

The gross aspects are not peculiar. The individual lesions are covered by smooth skin which is more or less fixed to the underlying nodule. The majority of these cysts are 0.8 to 1.5 cm. in diameter, although some very large cysts have been reported, as that of Chiari⁴ which measured 6 by 13 cm. They may be slightly blue or yellow. The contained material, made up of dead, exfoliated, keratinized cells, has the same flaky consistency as the material in epidermal cysts, but the addition of sebaceous secretion sometimes adds a waxiness or milkiness, and some cysts are described as containing an oily fluid.¹ Like epidermal cysts, they are subject to infection and are often obliterated by inflammation or fibrosis. Malignant change probably does not take place.

We have four follicular cysts in our material.

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B. CYSTIC BENIGN TUMORS OF FAMILIAL NATURE

"EPITHELIOMA ADENOIDES CYSTICUM"

Cystic epithelial tumors of the skin are very common. They may be divided into two main groups: those which are multiple, with certain clinical and histologic characteristics suggesting a congenital origin;³⁴ and those which are primarily tumors of middle age and may be classed with the nonkeratinizing tumors. There is no sharp dividing line between the two groups either on a clinical or a histologic basis. Nonkeratinizing epithelial tumors of the skin, of which the basal cell carcinoma is the frequent form, are prone to cystic degeneration of both the tumor and the stroma.²¹ Such tumors have often been confused with the true cystic tumors of sweat glands. It is too seldom recognized that tumors of entirely diverse histologic structure may present identical clinical features. For example, the common epithelial tumors of the skin, epidermoid carcinoma and basal cell carcinoma, are not always distinguishable clinically even by experienced observers. In this paper we have to deal with a similar problem, *viz.*, two groups of skin appendage tumors that are often confused clinically, but are histologically distinct.

The clinical condition under discussion, relatively rare, is characterized by multiple small tumors, averaging 0.5 cm. in diameter, usually scattered either over the chest and axillae or on the central part of the face and lower eyelids, but sometimes in both locations.⁴⁰ The gross lesions are discrete domed nodules, colorless, slightly discolored, or distinctly yellow, covered by smooth intact epidermis. This syndrome is usually, but not always, familial and occurs more often in females, especially at puberty.

Those who believe the process can be subdivided clinically state that the tumors occurring on the face are epithelioma adenoides cysticum, and those occurring primarily on the chest are syringoma, although the gross appearance of the lesions is similar, regardless of locality.

Only a very few microscopic examinations have been possible in proportion to the number of lesions on a given person. As a result, we know relatively little about their structure. There appear to be at least two histologically distinct tumors that may have these clinical and gross features. One resembles the duct of a sweat gland and the other a tumor of hair follicle.

The tumor of sweat duct type is said to be located primarily on the chest. It is less apt to be familial and is said to be radiosensitive. The tumor resembling hair follicles, on the other hand, is described as a distinctly yellow tumor⁴⁵ primarily on the center of the face and lower eyelids, that is radioresistant,¹⁰ and is familial with a few exceptions.^{3, 13, 26, 43} The tumors have been described in several members of a single generation and in successive generations.^{7, 13, 31, 39, 41}

The duct type of tumor^{19, 42} (syringoma) was discussed with tumors of sweat glands. We will describe briefly the tumors of hair follicles as seen in this condition. The microscopic structure is essentially that of a tumor of hair follicle,^{20, 23, 27, 32, 39, 44, 46} in which there is cystic degeneration and also some proliferation of the matrix. The follicle and the matrix cells may retain their specific characteristics, with a typically benign structure, although there may be transitions to hair matrix carcinoma. The tumors have been ascribed to the fully differentiated hair follicle,²⁰ lanugo hair,⁶ or undifferentiated anlagen²³ which are stimulated to growth at puberty.²² Such a sequestration of epithelium seems most likely to occur along body fissures²⁹ but many tumors cannot be accounted for in this way.

Unfortunately this tumor has never been given a definitive name. The least controversial of the terms by which it has been known is Brooke-Fordyce disease. Although it is now most often called epithelioma adenoides cysticum, there are two objections to this term. It is too vague: all nonkeratinizing epitheliomas tend to become cystic. And it has been used for a loosely defined clinical entity which may result from enlargement of sebaceous glands³⁶ as well as tumors of sweat glands and of hair follicles or basal epithelium.^{9, 16} It does not connote a single clearly defined pathologic process, and has been used for quite unrelated conditions.^{8, 38} Nevertheless, epithelioma adenoides cysticum as a descriptive term for multiple benign cystic lesions of hair-follicular origin is sufficiently well established to use so long as it is assigned to this specific lesion.

By no means has there been general accord as to the histologic structure or proper interpretation of the syndrome of multiple benign epithelial tumors.¹⁸ This is well illustrated by the differences of opinion on some of the early cases.^{2, 4, 12, 37, 45} The histologic structures of basal cell carcinomas, hair matrix carcinomas, and sebaceous adenomas have all been described in this syndrome of benign familial tumors of the face and chest.^{12, 15, 24, 25, 30, 46} Other congenital tumors such as the so-called cylindroma of the scalp may also be present.⁵ It is well to remember that benign skin tumors, as nevi, neurofibromas, lipomas, tumors of sweat glands, tend to be multiple; and also that multiple epithelial cutaneous tumors usually have an organoid structure and are for the most part nonkeratinized and benign. Considering the embryologic relationship of the epidermis and cutaneous appendages, a congenital dystrophy of dermal structures might well yield a variety of unusual epithelial structures. In only rare instances have any of these tumors been reported as clinically malignant.¹

The following terms have been applied to the general group of benign familial tumors which we have described in this paper:

1. Benign epitheliomata with colloid degeneration.³⁵
2. Epithelioma adenoides cysticum.⁶ (This is the term most widely accepted.)
3. Multiple benign cystic epithelioma.¹²
4. Tricho-epithelioma papillosum multiplex.²⁰
5. Acanthoma adenoides cysticum.⁴⁵
6. Hydradenome éruptif.¹⁹
7. Multiple cystic symmetric nevi.⁴⁷
8. Tricho-epithelioma papillosum with syringocystadenoma.²⁸
9. Cystic basocellular epithelioma.¹⁷
10. Naevus follicularis.³⁹
11. Brooke's tumor.¹⁴
12. Adenoid cystic epithelioma.¹¹

Treatment has not been successful since the multiplicity of lesions usually eliminates surgery and the tumors do not react uniformly to irradiation.^{10, 30} It has been said that growth may be retarded by radiant energy and the partly degenerated cystic tumor expressed or excised.³⁹ However, the resulting scar may be just as objectionable as the tumor. If there are only a few tumors, either surgical excision or carbon dioxide snow or electrodesiccation may be satisfactory.

Malignancy has been described following irritation or improper treatment. Among the 116 cases in the literature which were reported in detail, there were 5 cases of secondarily arising carcinomas, an inci-

dence of 4.3 per cent. The carcinomas are usually of basal cell type,²⁴ but Gordon¹⁴ reported an intra-epidermal epithelioma.

Summary. We use the term epithelioma adenoides cysticum for a benign lesion of hair follicles which is grossly indistinguishable from syringoma and, like it, multiple and familial. The so-called syringomatous type is properly classified with tumors of sweat glands.

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C. CALCIFIED CYSTS AND CALCIFIED EPITHELIOMAS

The number of reports which have appeared on "calcified epithelioma" seem out of proportion to its clinical significance. As Ewing⁴ has pointed out, minor focal calcification occurs frequently in all kinds of epithelial tumors and probably represents a regressive process usually associated with sluggish growth and necrosis. A marked degree of calcification is less common and complete calcification of a tumor rare. Tanasescu and Balan¹⁹ have reported a large series of calcified cutaneous epitheliomas.

We interpret most of the descriptions and photomicrographs of the reported calcified epitheliomas as compatible with calcification of the contents of an epidermal cyst.^{2, 7, 9} Nevertheless, the true calcified epithelioma has been generally recognized and carefully described in the literature. "Calcified epithelioma" was early made familiar by Malherbe,¹⁴ who described calcified tumors of sebaceous glands and later noted a similar calcification of cysts of sweat glands.¹⁵ Unna²⁰ pointed out that tumors of the scalp and face are especially apt to calcify. The following list shows the different types of tumors which have been considered important in the formation of calcified epitheliomas:

1. Nevi (*i.e.*, congenital tumors)^{5, 6, 8, 10, 11}
2. Sebaceous gland tumors, either from anlagen or differentiated glands^{13, 14, 18}
3. Epidermal cysts⁷
4. Epitheliomas arising from epidermal cysts⁷
5. Calcified carcinomas^{1, 3, 7, 12, 21}
6. Calcified basal cell tumor²

Ossification of calcified tumors may occur¹⁷ and Musger¹⁶ described a case of basal cell carcinoma in which osteogenic sarcoma arose.

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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 95

- FIG. 1. Epidermal inclusion cyst with chronic inflammation and foreign body giant cell reaction. Phosphotungstic acid hematoxylin stain. $\times 150$.
- FIG. 2. Sebaceous cyst with sebaceous gland carcinoma developing from one margin. Hematoxylin and eosin stain. $\times 12$.
- FIG. 3. Dermoid cyst of skin. Multiple hairs are present in the cyst. Phosphotungstic acid hematoxylin stain. $\times 19$.



Warvi and Gates

Cysts and Cystic Tumors of the Skin



DIETARY ULCERS OF THE ESOPHAGUS OF THE RAT *

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In the course of determining the long-term effects of a deficient rice diet on white rats, peculiar lesions of the esophagus were noted in a number of animals. The most striking and apparently the farthest advanced alterations were focal ulcerations of the mucosa accompanied by various stages of penetrating inflammation. Other changes included mucosal hyperkeratosis and edema of the submucosa, atrophy and fibrosis of the muscularis, and dilatation of the organ itself, at times without demonstrable cause. Essentially similar lesions of the fore-stomach of the rat were described by Pappenheimer and Larimore¹ in 1924. These authors implicated a mechanical as well as a dietary factor. Since the morphology of the lesions in their group as well as in mine suggested the participation of a mechanical factor, it was necessary for me to exclude this in order to establish a dietary etiology for the rat esophageal ulcers. The production of the esophageal lesions and their morphology in *Group I*, and their prevention by a largely synthetic vitamin diet in *Group II*, are described in this paper.

METHODS AND RESULTS

Group I

Seventeen white rats of the Osborne-Mendel strain, between 2 and 3 months of age, were placed on a diet of fresh, finely ground, unpolished Texas rice mixed with cottonseed oil in the ratio of 20 cc. of oil to 1000 gm. of rice. This diet was supplemented with a small slice of fresh carrot, each rat receiving daily approximately 1 gm. The rats were fed the diet and given water *ad libitum*. (See Sugiura and Rhoads² for a discussion of this diet.)

Only one rat died a significant time before the experimental period of 6 months was over. Three died a week previous to this time. All of the animals gained some weight during the experimental period, but many of them lost weight after the first few months. At the end of the experimental period two weighed less than their original weight (Table I). Two of the rats (39L16 and 31L61) were fed the diet for 8 months before being killed. One of these had multiple typical esophageal ulcerations, the other showed mucosal thickening and questionable hyperkeratosis.

* Received for publication, December 10, 1942.

TABLE I
Experimental Data for Group I

Rat no.	Esophagus	Born	Diet began	Killed	Beginning weight	Maximum weight	Dead weight
					(gm.)	(gm.)	(gm.)
39L16	++	9-2-41	11-12-41	7-7-42	140	180	145
52L20	++	9-10-41	11-12-41	5-31-42	145	180	154
40L16	++	9-2-41	11-12-41	5-24-42*	135	185	122
23L63	++	7-22-42	10-9-41	3-25-42	210	280	210
17L63	++	7-25-42	10-9-41	3-25-42	157	210	180
19L51	++	7-14-42	10-9-41	3-19-42*	164	255	255
24L60	++	8-23-42	10-21-42	3-25-42	133	205	210
41L16	++†	9-2-41	11-12-41	5-26-42	160	200	145
32L61	+	8-29-41	11-12-41	5-24-42*	130	190	122
29L61	+	9-29-41	11-12-41	4-11-42*	180	240	180
38L16	+	9-2-41	11-12-41	5-31-42	120	130	100
14L51	+	7-14-42	10-9-42	3-25-42	130	175	160
3L3	±	7-26-42	10-9-42	3-25-42	148	195	180
31L61	±	8-29-41	11-12-41	7-7-42	160	210	205
16L22	o	7-22-42	10-9-42	3-25-42	213	285	260
4L3	o	7-26-42	10-9-42	3-25-42	148	205	195
5L3	o	7-26-42	10-9-42	3-25-42	140	190	150

++ Ulceration and hyperkeratosis.

+ Hyperkeratosis. 38L16 showed mucosal erosion; 32L61 showed dilatation of esophagus in addition to hyperkeratosis.

* Died.

† Ulceration located in forestomach instead of in esophagus.

Some of the rats had a moist, reddish brown discoloration around the nose, and many developed a coarse, shaggy coat. A few of the animals lost hair on the abdomen and in the groin and axilla. The conspicuous lesions of the ears and paws described by Antopol and Unna³ as characterizing B₆ and pantothenic acid deficiency were not encountered. Neither was their general appearance suggestive of vitamin A deficiency. Eye signs, as well as infections in the cervical region or urinary tract, were absent except for some blepharitis in one animal. The rats increased in size but most of them appeared to be malnourished at the end of the experimental period.

No consistent abnormality was noted in any organs other than the esophagus. The esophageal lesions, when fully advanced, were quite striking (Figs. 1 to 5). Seven of the 17 rats had ulcers in the lower esophagus and 1 had an ulcer of the forestomach. The ulcers were occasionally confluent and in 1 rat extended above the mid-esophagus. The larger ones were of the penetrating type, as attested by contiguous inflammation of the liver, lungs and pleural cavity. The mucosa about the ulcers was wrinkled with white islets and ridges of leukoplakia. Small ulcers were noted in these leukoplakial spots, and the edges of the large ulcers were raised, white and glistening (Fig. 3). The esophagus was dilated to varying degrees about the ulcers and above them. Masses of rice were frequently found impacted in these sac-

cular dilatations. Digital palpation of the rice disclosed a distinct sharpness of the granules.

In rat 32L61 no ulcers accompanied the rather extensive dilatation of the lower and mid-esophagus. In rat 40L16 advanced ulceration was present in addition to the sacculation of the organ (Fig. 1).

All of the esophagi were sectioned, and from most animals sections of the lung, forestomach, renal pelvis, liver and bladder were taken. The large esophageal ulcers were essentially similar. The squamous epithelium at their edges was thickened in all layers and usually was hyperkeratinized (Fig. 12). Numerous mitoses were found in the basal layers of the adjacent squamous epithelium. The granulation tissue at the ulcer bases was rich in capillaries and was crowded with eosinophils and polymorphonuclears and contained some lymphocytes, plasma cells and macrophages. Fibroblastic activity was advanced and edema of the submucosa was conspicuous. In many ulcers, fused eosinophilic collagen and disintegrating muscle fibers formed the base, an appearance not unlike that seen in peptic ulcers and sometimes attributed to contact with gastric juice. Masses of bacteria were seen in the large necrotic ulcer craters. In one rat there were sizable varicosities of the deep esophageal veins beneath a zone of mucosal erosion and ulceration.

The small ulcers were likewise surrounded by hyperplastic hyperkeratinized squamous epithelium (Fig. 11). In two rats it appeared as though the thickened corneum and superficial malpighian layers were being shed off or pulled off into the lumen. In one animal (Fig. 10) a focus of acute inflammatory cells in the submucosa, and in another edema of the papillae, accompanied these superficial changes, to give evidence that the process was certainly not entirely, and possibly not primarily, a mucosal one.

In animal 41L16 a punched-out ulcer was noted on routine microscopic study of the forestomach, whereas neither gross nor microscopic ulceration was found in the esophagus. In addition there was nearby a verrucous focus in the mucosa formed by hyperplastic cells of the stratum germinativum which grew down to the muscularis mucosae obliterating the submucosal papillae (Fig. 13). The appearance and location of these lesions is reminiscent of those encountered by numerous authors in vitamin A deficiency.

Only one animal in the series exhibited saccular dilatation of the esophagus without demonstrable ulcerative changes in the mucous membrane. Rat 32L61 was peculiar in this respect (Fig. 1) and there is some doubt that ulcerative changes were not present in the mid-esophagus of the animal. Sections of the mid-esophagus showed ad-

vanced thickening and fibrosis of the muscularis. The muscularis at this level was heavily infiltrated with polymorphonuclear leukocytes, eosinophils and lymphocytes, and the muscle fibers were largely replaced by proliferating fibroblasts. Examination of the overlying squamous epithelium revealed thickening but no ulcerative defect.

Somewhat similar changes were noted in the mucous membrane of rat 29L61 (Fig. 7) although no dilatation accompanied them. In the lower esophagus of this animal the squamous lining was in some places atrophic, while in others it was hypertrophied, heaped up and papillary. The rete pegs were separated by delicate edematous connective tissue papillae in which scattered plasma cells and dilated capillaries were noted (Fig. 8).

Two additional animals, 14L51 and 38L16, exhibited definite hyperkeratosis (Figs. 6 and 9). Beneath this, scattered polymorphonuclear leukocytes lay in a slightly edematous submucosa.

Of the remaining five animals, three had unequivocally normal-appearing esophageal mucosae. In the remaining two the mucosa in the lower end was hypertrophied and covered with a stratum corneum equal to one-half the entire mucosal thickness. Kullmann,⁴ after study of the rat esophagus, stated that the cornified layer usually comprised one-third of the thickness of the mucosa. For this reason rats 31L61 and 3L3 were considered borderline cases. None of these five animals exhibited submucosal changes.

Group II

A group of 16 rats similar in age, sex and weight to those of group I was placed on the same deficient diet for a period of 5 months. Synthetic vitamins were added to this diet in the proportions noted below * for a period of 1 month, then the animals were sacrificed. The amounts supplied were well in excess of the vitamin dietary requirements for the rat as determined by Richardson, Hogan, Long and Itschner⁵ and contained, in addition, nicotinic acid and vitamin K. The rats ate noticeably increased amounts of the vitamin-supplemented diet, and underwent an average weight gain of 76 gm. Prior to the addition of vitamins four animals developed ulcers of the skin at the base of the tail, one an ulcer of the penis, one an ulcer over the shoulder, and one an ulcer of the scrotum. Upon the addition of the vitamins all of the

* The vitamins are expressed in mg. per 100 gm. of diet: α -tocopherol, 5; thiamine hydrochloride, 1.6; riboflavin, 3.2; pyridoxin hydrochloride, 2.4; calcium pantothenate, 2.0; choline chloride, 800; nicotinic acid, 2; synthetic vitamin K, 0.02. Two gm. of cod liver oil per 100 gm. of rice furnished vitamins A and D. Four gm. of salt mixture (no. 2, U. S. P.) per 100 gm. were also added. Merck and Co. kindly furnished the first seven synthetic vitamins.

ulcers but two healed completely and these two nearly healed. When the animals were sacrificed at the end of the experimental period of 6 months (1 month after the vitamins were added) they appeared healthy. Careful examination of all internal viscera showed no abnormality. Microscopic sections of the parotid gland, trachea, kidney pelvis, bladder, esophagus, stomach, forestomach and cecum were made. Three of this group showed slight hyperkeratosis of the lower esophageal mucosa with underlying round-cell infiltration, and one showed similar foci in the forestomach. No ulcers were noted. Six of the group exhibited multiple minute calcium foci in the tubular epithelium of the kidneys. In six the sections were entirely negative. Microscopically there appeared to be nothing specific about the inflammatory changes occurring at the sites of skin ulceration.

DISCUSSION

In reviewing the lesions associated with the various dietary deficiencies one is impressed with the similarity between the lesions here described and those of vitamin A deficiency. The hyperkeratosis associated with esophageal ulcers seems reasonably characteristic of mucosal changes in A deficiency. After a search through a considerable number of articles⁶⁻¹³ dealing with the experimental pathology of this condition, however, I was able to locate none describing esophageal ulcers and only one mentioning esophageal hyperkeratosis. Tilden and Miller,¹⁴ in a study of the effects of a low vitamin A intake on 11 monkeys, noted 3 in which keratinization of the esophagus was discernible and 1 in which it was questionable. The same condition was reported in 5 of a group of 17 Chinese suffering from a dietary deficiency, most likely A, by Sweet and K'ang.¹⁵ The absence of other more obvious signs of vitamin A deficiency in my animals indicates that a full-fledged deficiency did not exist. Nor would one expect it in animals receiving even a small amount of carrot daily. It is possible, however, that a partial deficiency did exist and that its effects became cumulative over a long period to become manifest spontaneously or as a result of some local phenomenon—in this case mechanical irritation of the ground rice diet.

Denton's¹⁶ descriptions of the lesions of experimental black tongue in dogs set forth some features which my rats exhibited. He characterized the condition of black tongue as a membranous necrosis of the mucosa of the mouth, pharynx, esophagus, intestines, and skin of the scrotum. No oral lesions occurred in my group, but two rats had duodenitis, and a number of rats in group II exhibited skin lesions at the base of the tail and on the scrotum. The focal nature of the ulcera-

tions in my group stands in contrast to the diffuse gastrointestinal inflammation of black tongue.

Erosions of the gizzard in chickens as described by McFarlane, Graham and Hall¹⁷ and by Dam¹⁸ were thought by Almquist and Stokstad¹⁹ to be caused by a deficiency of a fat-soluble factor not identical with any of the known vitamins. Morphologic studies by these authors are not sufficiently detailed to allow a comparison with the lesions of my rats.

In group II of my series it seems significant that no esophageal ulcers were present after the addition of a full vitamin supplement for only 1 month. Also indicative of a more adequate dietary state is the prompt gain in weight and healing of the skin lesions. Except for the cod liver oil, very little in the way of caloric value was added. The finding of hyperkeratotic mucosal changes in the four animals after the addition of vitamin supplements suggests that a deficiency did exist or was developing prior to the addition of the vitamins.

SUMMARY

1. Lesions of the esophagus of the rat, including mucosal ulceration, hyperkeratosis, various stages of inflammation, and dilatation with muscle atrophy and fibrosis have been described.
2. These lesions appear to be of dietary origin.

I wish to acknowledge my indebtedness to Robert McLemore for technical assistance.

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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 96

FIG. 1. Fusiform dilatation of the lower esophagus. Rat 40L16 showed mucosal ulceration, but only hyperkeratosis was noted in rat 32L61. $\times 1$.

FIGS. 2, 3 and 4. Gastro-esophageal junction. There is ulceration with accompanying hyperkeratosis. The lesions found in rats 23L63, 39L16 and 19L51, respectively, are represented. $\times 2$.

FIG. 5. Penetrating ulcer of the lower esophagus in rat 17L63. $\times 2$.

340L16

332L61



1



2



3



4



5

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Dietary Ulcers of the Esophagus

PLATE 97

- FIG. 6. Rat 14L51. A focus of hyperkeratosis in the lower esophageal mucosa. The rete pegs are thickened. Eosinophils and lymphocytes are scattered beneath the muscularis mucosae. $\times 48$.
- FIG. 7. Rat 29L61. A focus of hyperkeratosis from the lower esophagus. Edematous submucosal papillae separate thickened rete pegs. Dark strands on the surface of the keratinized layers are made up of small granules resembling rice. $\times 48$.
- FIG. 8. Rat 29L61. A wide zone of edema surrounds a delicate capillary of a papilla. Occasional polymorphonuclear leukocytes and lymphocytes are observed. Fine rice granules can be seen in the thickened stratum corneum. These are smaller than the keratohyaline granules. $\times 240$.
- FIG. 9. Rat 38L16. Desquamation of the corneum and superficial malpighian layers caused widespread erosion but no ulceration in the lower esophagus. $\times 48$.
- FIG. 10. Rat 39L16. Similar mucosal fragmentation is seen here, but ulceration is beginning and an exudate, made up chiefly of eosinophils, pushes the muscularis mucosae upward. $\times 48$.
- FIG. 11. Rat 24L60. A small pregastric ulcer is seen extending down to the muscularis mucosae. The vacuolar changes in the adjacent squamous epithelium were not conspicuous in other animals. $\times 48$.
- FIG. 12. Rat 19L51. The edges of a large penetrating ulcer are seen, which in its center extends through the muscularis. Necrotic debris, bacteria and rice granules cover the ulcer base. $\times 48$.
- FIG. 13. Rat 41L17. Focal verrucous thickening of the squamous epithelium of the forestomach. Elsewhere in the forestomach was an ulcer similar to that shown in Figure 11. $\times 48$.



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Dietary Ulcers of the Esophagus

THE CO-INCIDENCE OF PRIMARY CARCINOMA OF THE LUNGS AND PULMONARY ASBESTOSIS *

ANALYSIS OF LITERATURE AND REPORT OF THREE CASES

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A review of the literature on pulmonary asbestosis by Egbert in 1935 resulted in the publication¹ of 25 cases supported by anatomic study, thus reflecting the increasing importance of the disease.

In 1935, Lynch and Smith² reported *primary carcinoma of the lungs associated with asbestosis*. Four years later the same authors³ described 2 additional cases of their own and collected 5 more from the literature,⁴⁻⁷ bringing the total to 8. A recent survey revealed 8 more cases, which are presented in Table I. Three more instances are contributed in the following report, thus bringing the total number to 19.

Pulmonary asbestosis has been diagnosed in this laboratory according to the following criteria:

1. Fibrosis of the lung.
2. Presence of asbestosis bodies.^{8, 9} Kühn¹⁰ presented photographs of asbestosis bodies obtained by the electronic microscope, enlargement $\times 20,800$.

REPORTS OF CASES

Case 1

The patient (autopsy no. 1705), a white male, 45 years old, was exposed to dust in an asbestos factory for 5 years. For at least 1 year before his death he had complained of gradually increasing weakness and dyspnea. This forced him to stay in bed for 6 weeks prior to his admission to the New Haven Hospital. The clinical impression at this time was diaphragmatic pleurisy and compression of the lung on the right side, possibly tuberculous in origin. Generalized arteriosclerosis and paroxysmal tachycardia were found as complicating factors. The patient expired after a short stay in the hospital.

Only those portions of the post-mortem findings important for this report are included.

Gross Description of the Lungs. Each lung weighed 700 gm. The surface of the right lung was covered by white, firm adhesions which bound it to the parietal pleura. Only a few small areas of the visceral pleura were free from adhesions. These areas were pink, mottled with the usual amount of black subpleural pigment. The diaphragmatic surface exhibited a large number of firm, white, opaque nodules, 2 to 5 cm.

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TABLE I
Collected Cases of Primary Carcinoma of the Lung Associated with Asbestosis

Number and author	Year	Sex and age	Occupation in asbestos industry	Duration of exposure	Freedom from exposure before death	Nature of tumor	Primary site	Metastases
* 1. Lynch and Smith ³	1935	M., 57	Weaver	21 yrs.	4 mos.	Squamous cell	Right lower lobe	Many nodules in right lower lobe
* 2. Gloyne ⁵	1935	F., 35	Spinner	8 yrs.	9 yrs.	Squamous cell	Right upper lobe	Pleura
* 3. Gloyne ⁵	1935	F., 71	Mattress and opening department	19 mos.	15 yrs.	Squamous cell	Right lower lobe	None
* 4. Egbert and Geiger ⁴	1936	M., 41	Weaver	17 yrs.	2 yrs.	Glandular	Left lower lobe	Widespread
* 5. Gloyne ⁵	1936	M., 59	Packer	10½ yrs.	? mos.	Oat cell	Left lower lobe	Left upper lobe; pleura
* 6. Nordmann ⁷	1938	F., 35	Spinner	7 yrs.	9 yrs.	Squamous cell	Left lower lobe	Liver, kidney
* 7. Nordmann ⁷	1938	M., 55	Pre-spinning assembly	7 yrs.	12 yrs.	Squamous cell	Left lower lobe	Widespread
* 8. Lynch and Smith ³	1939	M., 50	Weaver	13 yrs.	3 yrs.	Squamous cell	Right lower lobe	Pleura, mediastinal lymph nodes
9 and 10. Koelsch ¹¹	1940	No details known. Oral communication of Domenici, quoted by Koelsch.						
11. Linzbach and Wedler ¹²	1941	No details known. Oral communication of Bohne, quoted by Linzbach and Wedler.						
12. Linzbach and Wedler ¹²	1941	M., 61		At least 3 yrs.	Not exactly known	Squamous cell	Right lower lobe	None

TABLE I—(Continued)

Number and author	Year	Sex and age	Occupation in asbestos industry	Duration of exposure	Freedom from exposure before death	Nature of tumor	Primary site	Metastases
*13. Holleb and Angrist ¹³	1941	M., 52	Pipe insulator	25 yrs.	9 wks.	Squamous cell	Right upper lobe	Mediastinal nodes, kidney, adrenal
*14. Holleb and Angrist ¹³	1941	M., 58	Pipe insulator	25 yrs.	10 yrs.	Oat cell	Right lower lobe	Widespread
15. Desmeules and others ¹⁴	1941	M., 57	Machine adjuster	25 yrs.	1 mo. (?)	Alveolar cell	Left lung	Pleura
16. Desmeules and others ¹⁴	1941	M., 50	Bagger	22 yrs.	4 mos.	Squamous cell	Right lung	Pleura
17. Homburger	1942	M., 45	Not known	5 yrs.	1 yr.	Squamous cell	Right lung	Diaphragm
18. Homburger	1942	M., 43	Not known	20 yrs.	17 mos.	Anaplastic	Left lower lobe	Pleura
19. Homburger	1942	F., 49	Not known contact with asbestos	Not known	Not known	Squamous cell	Right lung	Liver, adrenal, stomach, hilar lymph nodes

* Included in previous tabulations by Nordmann, Angrist and Holleb.

in diameter with scattered zones of necrosis. Similar masses were attached to the parietal wall of the pericardium. The lung was firm and only slight crepitation could be elicited. This was particularly true at the angle between the diaphragmatic and the mediastinal surface. On section this latter portion was white and opaque. Smaller nodules were seen in the lung parenchyma near the interlobar fissures. The color of the remaining lung parenchyma was mottled dark red and black. The cut surface was moist and exuded red frothy fluid upon slight pressure. Some of the black zones were firmer than the remaining parenchyma. In the left lung similar black, firm, fibrous nodules and a red, moist cut surface were encountered. There were no white nodules in this lung.

Microscopic Description. The white, firm nodules seen grossly in the right lung proved to be squamous cell carcinoma with mitoses and pearl formation. The tumor involved the walls of the bronchi. It extended into the pleura where it was associated with much fibrous tissue and marked thickening. The lung not involved by tumor was well preserved. Some interstitial fibrosis was noted in these areas. The alveoli were filled with mononuclear cells. *Asbestosis bodies* were present in the lumina of alveoli, in the alveolar walls, in the tumor proper and in the interstitial fibrotic tissue of the lung as well as in the pleura. There was a chronic bronchitis but the epithelium of the bronchi was essentially unaltered. The exact point of origin of the tumor was not determined (Figs. 2 and 3).

Anatomic Diagnosis. Pigmentation and fibrosis of lungs (histologically, asbestosis); bronchiectasis; fibrous pleural adhesions; squamous cell carcinoma (probably carcinoma of bronchus) involving right lung, pleura, pericardium and thoracic wall; cardiac hypertrophy and dilatation; passive congestion of viscera; emaciation. Subsidiary: Cyst of thyroid gland; adenoma of pancreas.

Case 2

The patient (autopsy no. 3841) was a white male, 43 years old, who had worked in an asbestos factory for 20 years. Asbestosis bodies had been found in his sputum 17 months prior to his death. For several years before his death he had complained of pain in the back, radiating to the lower limbs. This was shown to be associated with rotation of the first lumbar vertebra and sacralization of the fifth lumbar vertebra. There had been also an incomplete fracture of the left femur. Generalized mottling throughout both lungs was observed at this time. When the patient began to complain of shooting pain in the lower back, ascending to the midback and radiating to the left side of the chest, further roentgenograms were made of the chest and a circumscribed shadow in the left lower lobe was interpreted as a lung tumor. In retrospect it was possible to see this shadow on earlier films. The tumor displaced the left main bronchus and the esophagus to the right. Exploratory thoracotomy and biopsy established the diagnosis of anaplastic carcinoma. The

patient became cyanotic after operation, developed pneumonia of the left upper lobe and expired.

Clinical Diagnosis. Pneumoconiosis (asbestosis); lung tumor (left lower lobe) with pressure on spinal root or metastases to spine.

Gross Description of the Lungs. The right lung was normal in size and shape. It was remarkably heavy, only slightly crepitant and held its shape when removed from the body. It was covered by a uniformly thickened, gray and translucent pleura. The cut surface was relatively smooth, finely granular, pale, slate-gray tinged with pink and mottled due to the presence of innumerable small, slate-gray polygonal areas bounded by gray-white connective tissue septa. In the lower lobe these areas were crepitant; the surface had a gray, more homogeneous appearance and was still firmer than that of the upper lobe. The walls of the smaller bronchi and bronchioles were thickened; a rather marked reddening of the mucosa of the larger bronchi was evident. In the posterior part of the left lower lobe a firm, irregular mass, measuring 5 by 6 by 7 cm., was encountered. Immediately beneath it lay the 8th and 9th intercostal nerves. It projected posteriorly and also medially from the surface of the lung. Posteriorly, the visceral and parietal pleurae were thickened and fused over the tumor in an area about 4 cm. in diameter, where the mass was covered only by delicate areolar tissue. Medially it projected a distance of several centimeters into the mediastinum, being adherent to the descending aorta and displacing the aorta and esophagus to the right. The left vagus nerve was embedded in the tumor mass for a distance of about 3 cm. at a point well below the cardiac plexuses.

No other mediastinal structures appeared to be involved. There were several large, soft mediastinal lymph nodes which, on section, presented a brownish black surface.

The cut surfaces of upper and lower lobes of the left lung resembled those of the right lung. The cut surface of the tumor was gray-white and marble-like with scalloped edges. It was closely associated with, and in one place apparently involved, the wall of a branch of the left main bronchus. Careful examination revealed no evidence of extension or metastasis to the nearby vertebrae or ribs.

Microscopic Description. Diffuse fibrosis was present throughout the lungs, not only in the peribronchiolar and perivascular regions but also in many of the alveolar septa. The alveoli were lined with cells which were almost uniformly swollen, prominent and in many cases detached from the wall. Many of the alveoli contained typical *asbestosis bodies* (Fig. 1). A few of them lay within the peribronchial or perivascular lymphatic tissue but the majority were in the alveolar spaces. They

were rarely free, but usually surrounded and sometimes partially phagocytized by large mononuclear cells and multinucleated giant cells. Scattered anaplastic cells with atypical hyperchromatic nuclei and abundant cytoplasm were present in the tumor mass. Numerous mitotic figures were seen. In other sections the cells were in cords arranged on a scanty connective tissue stroma. There was a tendency to form alveoli. In one area the tumor cells had grown in sheet-like masses, suggesting an epidermoid carcinoma. Branches of the vagus nerve were found firmly embedded in neoplastic tissue and the perineurium was invaded by carcinoma cells. Rare small asbestosis bodies were seen in the sinuses of a tracheobronchial lymph node in addition to many phagocytes loaded with anthracotic pigment (Fig. 6).

Anatomic Diagnosis. Pulmonary asbestosis; carcinoma of lung (left lower lobe), compressing 8th and 9th intercostal nerves and surrounding and compressing left vagus nerve; scar of recent thoracotomy; sero-fibrinous pleurisy (left). Subsidiary: Fibrous apical scars (bilateral); fibrous pleural adhesions (bilateral).

Case 3

The rather incomplete history of this white female (autopsy no. 5443), 49 years old, failed to reveal contact with asbestos. Nineteen months prior to death she began to note shortness of breath and dry cough. There was scanty mucoid sputum. As her condition grew worse x-ray examinations led to the diagnosis of pneumonia and pleurisy on the right. Thoracic taps revealed right hemothorax. Some days prior to death, examination of the thorax showed displacement of the heart to the left and dullness over the right chest except for paravertebral tympany. There was also wheezing anteriorly and posteriorly. Straw-colored fluid was repeatedly aspirated from the right chest.

Clinical Diagnosis. Carcinoma of the right lung with metastasis to the liver.

Gross Description of the Lungs. The right lung weighed 1050 gm. The upper lobe was atelectatic and covered by thick, firm, fibrous adhesions. Creptitation was impaired in the peripheral portions of the middle and lower lobes.

The lungs were cut on a mechanical slicer into slabs 2.5 cm. thick. The medial third of the upper lobe was replaced by firm white nodules of neoplastic tissue in which compressed blood vessels and bronchi were still recognizable. In the upper portions of the middle lobe the tumor extended along the bronchi and blood vessels leading to the periphery, and the mediastinal lymph nodes were enlarged by tumor. The lung parenchyma, where free of tumor, was pale gray. Its interstitium was markedly thickened, gray and translucent. The consistency was rubbery and in the upper lobe there were some yellow areas of localized softening about 1 cm. in diameter. The bronchi,

carefully examined, were found to be free of intraluminal obstruction. The mucosa was red and swollen. No ulceration could be seen. The walls of several bronchi were thickened and the lumina reduced. Analogous changes were found in the intrapulmonic vessels on this side.

The left lung weighed 360 gm. Its pleural surface was thin and translucent and free of changes. The parenchyma was air-containing; the color was gray in the upper and red in the lower lobe. There was the usual pattern of anthracotic pigment in the pleura and, on section, the parenchyma was dry and only a few droplets of blood escaped from the section of the lower lobe. All the bronchi had the usual caliber; their mucosa was intact and pink. There was no change in the blood vessels of this side. The hilar lymph nodes were free from tumor.

Microscopic Description. The tumor in the right lung was composed of dense nests of squamous cells surrounded by fibrous connective tissue. These tumor cells varied in size and had pyknotic nuclei. Many were in mitotic division. The cytoplasm was clear and pale.

The neoplastic tissue had replaced the lung parenchyma. There were also large areas of round cell infiltration and foci of breakdown of the tumor. The pleura was locally thickened by dense fibrous tissue. There was also interstitial fibrosis. There was a moderate amount of anthracotic pigment. Adjacent to the tumor the lung was atelectatic. In some areas, however, the alveoli were dilated and filled with small and large round cells or amorphous pink-staining material. Rod-shaped brown deposits were present in the interstitial tissue and also in some of the alveoli. These had the appearance of *asbestosis bodies* (Fig. 4). They were scattered throughout the lung parenchyma near the tumor and also in more remote regions of the lung.

Most of the bronchi showed inflammatory changes with round cell infiltration in the submucosa. The epithelium was of the usual type and in some places was lifted from the limiting membrane by the round cells. Most of the lumina contained similar mononuclear cells. In one instance remnants of bronchial epithelium were seen in the center of a tumor nodule. Here the lung parenchyma was entirely replaced by dense fibrous tissue which constituted the stroma of the squamous cell carcinoma. Pearl formation was observed. There was also a purulent polymorphonuclear exudate in some of the bronchi and in some of the larger bronchioles.

Anatomic Diagnosis. Pulmonary asbestosis (microscopic); purulent bronchitis and bronchiolitis; bronchogenic carcinoma (right lung); metastasis to hilar and preaortic lymph nodes, liver, adrenal and gastric mucosa; infarct in spleen. Subsidiary: Uterine fibroids; healed mitral endocarditis; nevus on abdominal skin.

DISCUSSION

In 4137 autopsies from 1918 to 1938 there were 45 cases of pulmonary carcinoma in this laboratory,¹⁵ an incidence of 1.08%. Asbestosis was diagnosed 8 times, silicosis 17 times. Pulmonary carcinoma was found in 4* of the 8 instances of asbestosis and twice in the 17 silicotic cases.

A similar coincidence of silicosis and cancer was found by Klotz¹⁶ (8%), but Vorwald and Karr¹⁷ could not confirm this. Because of the small series of cases, the value of these data is limited. Lynch and Smith² suggested that asbestosis is a predisposing factor in carcinoma of the lung. They stated that advanced asbestosis "may lead to bronchial epithelial metaplasia of a type encountered in other locations where cylindrical epithelium may give rise to squamous cell carcinoma," a point of view also shared by Linzbach and Wedler.¹² They cited the case of a negro who was thought to be asthmatic and who apparently died of heart failure. At autopsy pulmonary asbestosis, chronic bronchitis and bronchiectasis, acute lobar pneumonia and pulmonary atheromatosis were found, together with hypertrophy and dilatation of the heart. In the bronchi "foci of transformation of undenuded epithelium into stratified squamous form were found." It seems questionable whether this statement is of significance as an indication that asbestosis predisposes to carcinoma. During a study on bronchial metaplasia in this laboratory, metaplasia of the bronchial epithelium was found in only one instance of pneumoconiosis (case 5549, Fig. 10).

Metaplastic changes of bronchial mucosa were observed four times in a series of 44 consecutive autopsies, studied with special care from this point of view. Such metaplastic changes, illustrated in Figures 5, 7, 8 and 9, were seen in cases with various pulmonary lesions. None of these four cases presented any deposits of foreign material in the lungs.

These facts seem to indicate that neither from statistical calculations nor from purely morphologic studies is there any reliable answer to the question whether pulmonary asbestosis has to be considered as an etiologic factor in pulmonary carcinoma.

SUMMARY

A review of the literature on the association of pulmonary asbestosis and carcinoma revealed that there are at least 19 known cases (including the 3 herein reported) of asbestosis associated with primary pul-

* One of these cases was published previously by Egbert and Geiger.⁴

monary carcinoma. In this laboratory the association of the two conditions is remarkably high. In 8 cases of asbestosis there were 4 instances of primary pulmonary carcinoma.

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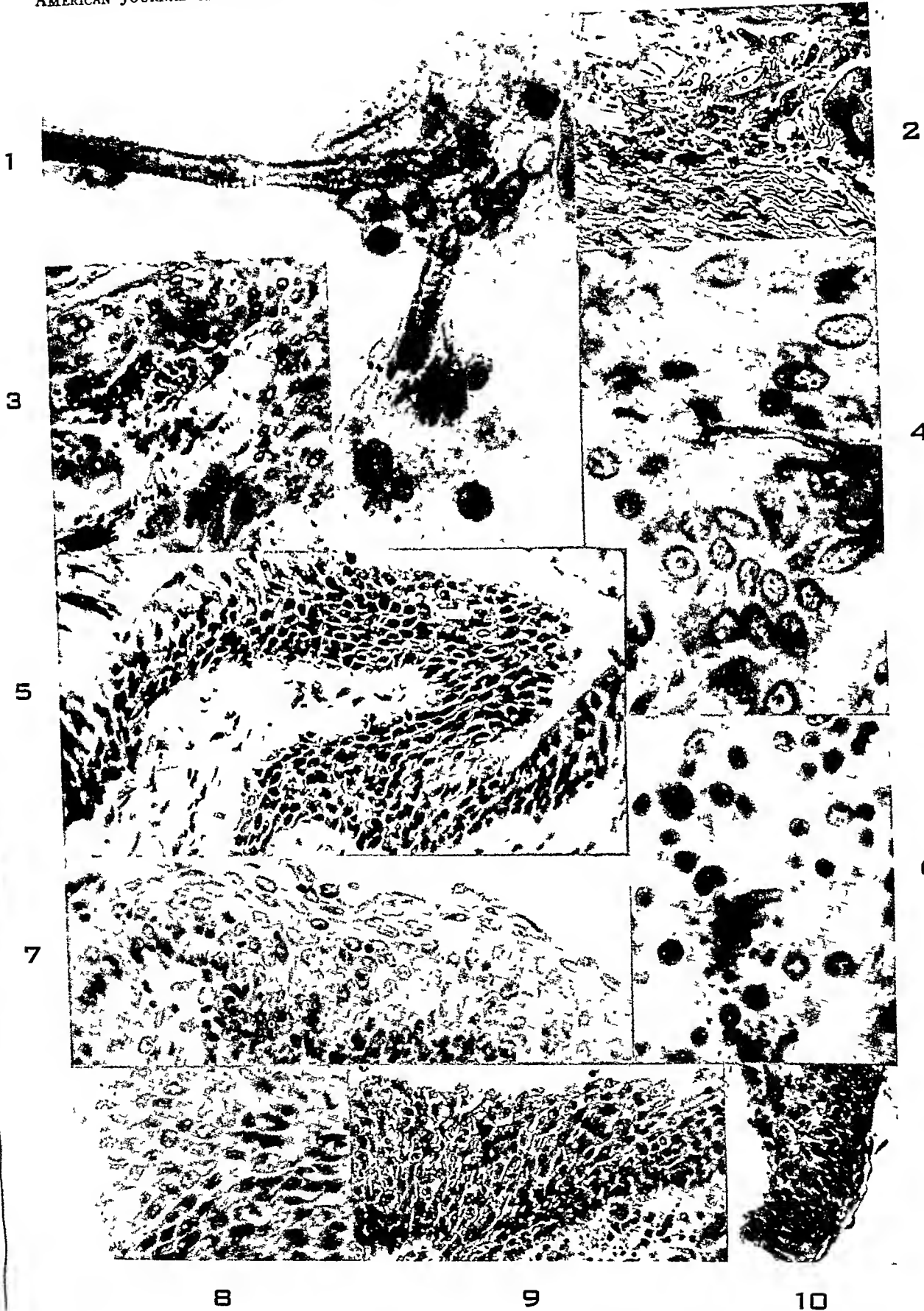
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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 98

- FIG. 1. Case 2 (A. 3841). Asbestosis bodies and phagocytes in pulmonary alveolar lumen. $\times 875$.
- FIG. 2. Case 1 (A. 1705). Nests of carcinoma cells, pulmonary fibrosis and numerous asbestosis bodies. $\times 220$.
- FIG. 3. Case 1 (A. 1705). Asbestosis bodies among carcinoma cells in lung. $\times 615$.
- FIG. 4. Case 3 (A. 5443). Asbestosis bodies and carcinoma cells in lung. $\times 395$.
- FIG. 5. White male, 36 years old (A. 5631). Death by violence. Pulmonary fibrosis. Bronchial metaplasia. $\times 220$.
- FIG. 6. Case 2 (A. 3841). Asbestosis body in sinus of peribronchial lymph node. $\times 655$.
- FIG. 7. White male, 57 years old (A. 5685). Healed endocarditis. Generalized arteriosclerosis. Pulmonary congestion. Bronchial metaplasia. $\times 370$.
- FIG. 8. White male, 40 years old (A. 5475). Caseous and ulcerative pulmonary tuberculosis. Bronchial metaplasia. $\times 370$.
- FIG. 9. White male, 66 years old (A. 5545). Pulmonary thrombo-arteritis, focal pneumonia. Bronchial metaplasia. $\times 220$.
- FIG. 10. White male, 43 years old (A. 5549). Chronic pulmonary abscesses, bronchiectasis, organizing pneumonia. (Questionable anthracosilicosis.) Bronchial metaplasia. $\times 220$.



Homburger

Pulmonary Asbestosis and Carcinoma

LOCAL MYELOPOIESIS IN MYELOID LEUKEMIA *

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Two explanations have to be considered for the genesis of extramedullary myelopoiesis. First, the hematopoietic foci may have developed *in situ* from local cells through metaplasia or prosoplasia by local transformation of unspecific cells into immature blood cells, which secondarily may enter the local vessels, thus reaching the circulating blood; second, production of the immature cells may have occurred in the bone marrow, with transportation by the blood stream to the involved organs, and formation of the foci by invasion of the perivascular tissue. Bloom¹ has suggested calling the first mechanism "heteroplastic" and the second "homoplastic." These short and significant terms are rarely used in the literature.

In general, the first theory is known by the phrase, extramedullary *myelopoiesis by local origin*. For the second, which has a basic similarity with the formation of deposits by malignant tumors, a special term, *colonization*, has been employed instead of using the term metastasis, to avoid a not fully proved analogy between leukemic blood cells and malignant tumor cells.

The mechanism of colonization itself shows certain remarkable differences from metastasis. In the choice of the host organ in which invading tumor cells settle and proliferate, in some special cases, biological affinity plays a deciding rôle. The ovary, for instance, is almost immune against cervical carcinoma growing nearby, but offers a particularly favorable soil for the cells of carcinoma of the stomach of distant origin. In general, in lymphogenic deposits, localization depends on mechanical conditions and topographical relations to the direct lymph flow. In hematogenic deposits, it is due to filtration of the tumor cells through narrow capillaries, as, for instance, in the pulmonary deposits of chorionic epithelioma. In "colonization" we see a definite predilection for certain organs. The immature blood elements are present in the circulating blood in numbers incomparably higher than malignant cells of any neoplasm ever are. They reach every organ of the body in a relatively high number, but settle in special organs only. This preference cannot be explained by narrow filtering capil-

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laries, since these are present in all organs. Also, the lymphatic connection exerts no influence, since colonization is accomplished only by the blood stream.

If we accept the hypothesis of colonization, we must face the fact that the organs selected by the immature blood cells for colonization, as the spleen, liver and, eventually, lymph nodes, are at the same time the organs of fetal hematopoiesis. This coincidence can only be explained by the auxiliary hypothesis, that in consequence of their function in fetal life, the tissues of these organs in extra-uterine life still retain a certain affinity for some elements of the blood. Special mechanical conditions may play a supporting rôle. Jaffé,² in his paper on extramedullary myelopoiesis in anemic mice, explained the myeloid foci he found in the liver either by colonization due to reduced speed of the blood in the wide capillary net of the liver or, possibly, to impaired circulation in the sinusoids, the lumen of which is reduced by Kupffer cells which are swollen in consequence of phagocytosis of red blood corpuscles. These mechanical conditions are responsible for the retention and the concentration of the circulating myelocytes. But in the same paper he stressed the relationship of Kupffer cells, sinus endothelial cells and reticulum cells with the elements of the bone marrow, and pointed out that these cells represent "familiar surroundings" for the circulating immature blood cells and thus facilitate their settling. Maximow³ described similar intravascular myeloid foci in the liver which he traced to stem cells deposited by the blood stream. However, such observations can serve only to explain the accumulation of myeloid cells inside of the sinusoids and contribute no clue for the histogenesis of the myeloid foci in the periportal fields.

In animal experiments the time relation plays an important rôle in making colonization probable. When the eosinophile cells appear in the tissues a few hours after a marked decrease in the same cells in blood, as, for instance, in the experiments carried out by Opie⁴ and by Homma,⁵ migration from the blood into the tissues or colonization is very probable. Most of these experiments, however, concern mature granulocytes only. For mature granulocytes active immigration by ameboid motility can be generally considered, whereas for immature ones only passive locomotion can be admitted. Moreover, eosinophile granulocytes, even when reaching full maturity, possess, as Sabin⁶ and Ringoen⁷ have pointed out, only a reduced active motility. However, in the experiments in which local accumulation of eosinophils by administration of bacteria or foreign animal proteins is accomplished, colonization by circulating myelocytes caused by chemotaxis seems admissible. The theory of colonization has found many authors to sup-

port it, from Ziegler⁸ and Helly⁹ in 1906 to Jaffé² in 1921, Lang¹⁰ in 1926, and Maximow¹¹ in 1927.

Local origin of extramedullary foci was described in 1905 by Pappenheim,¹² in 1916 by Herzog,¹³ in 1922 by Dieckmann,¹⁴ in 1931 by Naegeli,¹⁵ in 1926 by Ssyssojew,¹⁶ and by many others. It requires a different interpretation when it occurs with deficient erythropoiesis of bone marrow than when found with pathologically exuberant leukopoiesis in leukemia. In the first case it may be explained as compensation for the insufficient activity of the involuted bone marrow; in the second, the pathological agent which caused the proliferation of the bone marrow may have stimulated also the extramedullary undifferentiated elements of the mesenchyma, as far as they are apt and ready for metaplastic transformation into immature blood cells. In the first group the extramedullary myelopoiesis is an attempt at healing; in the second, it is a part of the pathological process itself. Changes of the first type were described by Brannan¹⁷ as fairly common in certain anemias of infancy and childhood. Erythropoietic foci are found not only in the organs of fetal hematopoiesis, such as liver, spleen, and lymph nodes, but large red masses of proliferating hematopoietic tissue can develop in the hilus of the kidneys and the falx cerebri.

It is interesting that in some of these cases the thymus, which physiologically is concerned with the production of lymphocytes only, changes into erythropoietic tissue—an ambiguity of function which parallels the activity of the red lymph nodes. In secondary anemia of adults, for instance in protracted bleeding after abortion, Brannan¹⁷ found erythropoietic tissue in organizing thrombi of the broad ligament.* These findings, together with the incidental formation of bone tissue in calcified thrombi as described by Wydler,²² prove the great and manifold prospective potencies of the fibroblasts which develop from the vascular wall and invade and organize the thrombi. The compensatory character of the extramedullary erythropoiesis becomes evident by the fact that in animal experiments it is demonstrable only in anemia provoked by agents injurious to the bone marrow, but not in cases where such factors are missing. A toxic incentive, which acts specifically on cells endowed with hematopoietic potencies, is yet to be isolated, as Lang²³ pointed out. It seems that, among the various types of interstitial tissue, the fat tissue possesses a special capacity for metaplastic change into hematopoietic tissue. Petri,²⁴ Wasser-

* Cone¹⁸ found in 68 of 250 necropsies bone marrow filling the intercostal veins which duplicated the bone marrow in the ribs. Like Lubarsch,¹⁹ Bunting²⁰ and Maximow²¹ in earlier observations, he attributed this bone marrow to embolism of myelogenous elements.

mann,²⁵ Jordan²⁶ and others have described multiple, newly formed, small hematopoietic foci in the fat tissue of various organs in cases of polycythemia, carcinosis and endocarditis. The hematopoietic islands develop from undifferentiated reticular elements which persist from fetal life in the fat tissue and may be classified among the so-called indifference zones of Schaper and Cohen.²⁷ Such pluripotent elements of low differentiation probably serve for the frequent changes between fatty and functional bone marrow. When, physiologically or pathologically, cells of these two types replace each other, it is not only done by reduction of one and *ex vacuo* proliferation of the other, but also by differentiation of these reticular pluripotent elements in one or the other direction and subsequent proliferation.

For the cells which give origin to extramedullary myelopoietic foci in leukemia, some authors have developed a broad conception which goes back to pluripotent qualities of embryonal mesenchyma. Dieckmann,¹⁴ in his paper on extramedullary hemopoiesis, has discussed this theory, which finds support in pathological investigations of Hueck²⁸ and in experiments carried out by Busse,²⁹ Maximow³ and others. Particularly, the observation that unspecific connective tissue, for instance from the heart valves, in tissue cultures may produce granulocytes or lymphocytes if stimulated, extends the faculty of hematopoiesis, as far as white cells are concerned, to a large field of unspecific mesenchyma. Modern conceptions in embryology, as represented for instance by Gruenwald,³⁰ are in favor of very little restriction in prosoplastic potencies, and some recent findings in pathology cannot be explained satisfactorily without such embryological conceptions. Heterotopic endometrium, as encountered in many locations within the peritoneal cavity far from the uterus, finds its explanation by Heim's³¹ theory that primarily not only the müllerian duct but the whole coelomic lining possesses the capacity to form endometrium. This potency is physiologically abolished everywhere outside of the müllerian duct and may be pathologically preserved in any portion of the peritoneal lining. If aroused later by some stimulus, local prosoplastic differentiation can give origin to islands of heterotopic endometrium, *i.e.*, of peritoneal endometriosis.

The investigators who support a more restricted and specific origin of local myelopoiesis agree that in general the distribution of the foci shows a close relation of the myelogenous tissue to the blood vessels. As far as such observations concern the development of leukemic blood cells from endothelial elements, particularly of the liver and spleen, they harmonize with the conception of those who adhere to the theory that one group of white blood corpuscles—the monocytes—physiologi-

cally develop from endothelial elements of liver and spleen. Hitherto, two types of white blood cells have been observed developing incidentally from Kupffer cells: the megakaryocytes and the monocytes. Downey, Palmer and Powell³² have described a case of atypical myelosis in a white woman of 56 years, with from 1900 to 3300 white blood cells per cubic millimeter, increasing to 10,400 1 month after splenectomy. Specimens obtained from the liver during splenectomy showed megakaryocytes developing directly from the reticular stellate cells of the liver sinusoids without going through the polykaryocytic stage. Bloom¹ succeeded in provoking similar changes artificially in rabbits by injections first with lithium carmine and subsequently with *Bacterium monocytogenes*. Transformation of Kupffer cells into monocytes was observed by Jaffé³³ in a Chinese male of 28 years who had developed an acute monocytic leukemia (3,650 white blood cells with 28 per cent monoblasts and 41 per cent monocytes) with hemorrhages of lips and gingiva and hemorrhagic extravasations in the mesentery, the myocardium and the pleura. Many of the monocytes of the blood gave a positive oxidase reaction. In the sections of the liver, the Kupffer cells, though still sessile and stellate, were packed with oxidase granules, as were some of the endothelial cells of the splenic sinuses. I have had the opportunity to observe a case which showed this change clearly.

REPORT OF CASE

E. R., a white man, 37 years old, suffered an attack of pain in the right lower quadrant of the abdomen 3 months before admission. A physician made a diagnosis of acute appendicitis, but blood count indicated a severe anemia, which contraindicated operation. The patient recovered from the attack but developed increasing weakness. Three days before admission he noted a dull, aching pain throughout the left side of the abdomen, which persisted. At the same time he started to bleed from the nose. He developed shortness of breath, which became progressively worse. Pulse was 120; respiration, 28; blood pressure, 100/60 mm. of Hg. Petechiae appeared in the left upper lid, and on the soft and the hard palate. On the tongue, at the tip, a 3 cm. irregular, firm, reddish yellow, tender mass was visible. Small, hard lymph nodes were palpable in the left supraclavicular fossa and in both axillae. The heart was slightly enlarged to the left. The spleen became very tender and was markedly enlarged. The liver also was tender and palpable two fingersbreadth below the right costal margin. The blood showed: hemoglobin, 37%; red blood cells, 1,900,000; microcytosis; white blood cells, 35,000 increasing to 64,000; neutrophile polymorphonuclears, 4%; lymphocytes, 13%; monocytes, 61%. The urine was cloudy; albumin, 4 plus; no sugar; many leukocytes and occasional red blood cells. Kahn and Wassermann tests were negative. The patient received several blood transfusions but became increasingly weaker and died 1 week after admission.

According to the family physician, 4 months before admission the blood findings were: hemoglobin, 82%; red blood cells, 3,400,000; white blood cells, 3,200; polymorphonuclears, 21%; eosinophils, 2%; bandforms, 2%; mononuclears, 5%; lymphocytes, 70%; blood platelets, 406,000; clotting time, 2 min.; bleeding time, 4

min. The stomach contents contained no free acid. Purpuric spots extended over the anterior surface, particularly of the legs. In spite of the predominance of the lymphocytes and because of the enlargement of spleen and liver, the marked anemia, and the mounting leukocytosis at this time, a subleukemic myelosis was diagnosed.

Autopsy. The following diagnoses were established by the autopsy: acute fibrino-hemorrhagic pericarditis; splenomegaly of 1470 gm.; sub-acute fibrinous perisplenitis and numerous anemic and hemorrhagic infarcts; myeloid infiltrations in heart, liver, kidneys and the distal half of the tongue; bilateral bronchiectasis; hemorrhages of both pleurae, of the mucosa of the stomach, of the epicardium, and of both kidneys in the subcapsular region. Microscopically the infiltration, which was of the same character in the enlarged lymph nodes, in the septa of the myocardium, in the pulp of the spleen, and in the periportal fields of the liver, consisted mainly of immature monocytes and monoblasts as well as of some myelocytes, metamyelocytes and polymorphonuclears. Many of the granulocytes were eosinophils. Most of the endothelial cells of the liver sinusoids presented a swollen and enlarged protoplasm. Some were of rounded contour and partially detached. Upon staining for oxidase the protoplasm of these cells presented numerous greenish black granules. Transitions from the fixed sinus endothelium cells into mononuclears were seen in great number, many of which were freely floating and filled the lumen of the sinusoids (Figs. 1 and 2).

This and Jaffé's³³ case prove that monocytes can develop from endothelial cells, at least in monocytic leukemia. For the normal this has been repeatedly suggested since the classical observation by Mallory,³⁴ who, as early as 1898, described desquamation of histiocytes from the vessels in the liver, spleen and lymph nodes of a patient with typhoid fever. Aschoff and Kiyono,³⁵ who distinguished two types of blood monocytes by the observation that after injection of collargol or lithium carmine some of the circulating monocytes do and some do not store the colloids, called the storing ones "histiomonocytes" and traced them back to desquamated reticulo-endothelial cells. Schilling,³⁶ in 1926, after having observed in endocarditis lenta as well as in mononuclear leukemia a continuous series of transitions between histiocytes and monocytes, established a triple system of white blood cells by adding to the two groups of granulocytes and lymphocytes the monocytes as a third independent group; that is, granulocytes originating from bone marrow, lymphocytes from the lymphatic tissue and monocytes from the reticulo-endothelial system. Similar origin of the monocytes has been assumed by Schittenhelm and Erhardt,³⁷ and by Büngeler.³⁸ On the other hand, Maximow³⁹ traced the monocytes back

to polyblasts and Bloom⁴⁰ suggested that they originate from lymphocytes only. He called the monocytes the physiological polyblasts of the blood, and described their development in the blood stream, especially in the sinuses of liver and spleen, by individual transformation of lymphocytes. Cases like Jaffé's³³ and mine prove the possibility that endothelial cells, or, more exactly, that Kupffer cells can be transformed into monocytes, at least in pathological conditions. Similarly, just as the granulocytes in cases of leukemia are different from the normal granulocytes, not only by being immature but by possessing some biologically obvious but morphologically latent characters by which they are related to neoplastic cells and differ from normal ones, so it is also likely that the monocytes of monocytic leukemia are pathological cells of different biological character compared with the physiological monocytes. This is made probable by the following microscopical observation. Whereas the monocytes of the normal blood, even when examined with the specially refined and accurate methods of McJunkin,⁴¹⁻⁴³ rarely present a few oxidase-positive granules only, in monocytic leukemia frequently almost all of the blood monocytes are loaded with great numbers of such granules. It seems that the production of oxidase-positive granules is a component of the pathological process, in the sense that a character which normally is present in low degree in some monocytes becomes strongly developed in all of them. This conception agrees with the findings of McJunkin, who proved the presence of some oxidase-positive granules in some of the Kupffer cells.

More frequent than the observations concerning the endothelial elements are those which prove the transformation of fixed elements of undifferentiated character, located in or adjacent to the wall of small blood vessels, into immature white blood corpuscles. This metamorphosis may pass through the phase of basophile hemocytoblast or may skip it, as observed by Bloom,⁴⁰ Maximow^{3, 11, 39, 44} and Lang.²³ The original cells are classified as adventitial histiocytes or, under the older term, as clasmatocytes or as perivascular reticulum cells. The low differentiation and high prosoplastic potencies of these cells have been definitely proved, although certain farguing theories, which are based upon these qualities, failed to meet general recognition, as, for instance, the hypothesis of Bostroem⁴⁵ which attempted to interpret the generalization of chorionic epithelioma not as metastases but as pluricentric transformation of adventitial elements in chorio-epitheliomatous tumor cells.

However, the occurrence of perivascular myeloid foci can be explained without the conception of special adventitial cells prone to undergo myelogenous metaplasia. Lang²³ offered the theory that the

incentive for the transformation, which is administered to the tissues by the circulating blood, is present in the highest concentration in and next to the vascular wall, and that as a result of this local high concentration the metaplastic immature blood cells are more frequent in this location. In spite of extensive experimental work, the question of the origin of the artificially provoked extramedullary myelopoiesis in animals has not been settled. Barnes and Sisman,⁴⁶ who, in co-operation with Furth,⁴⁷ successfully established criteria for differentiating myeloid leukemia from nonmalignant extramedullary myelopoiesis in mice, admitted that the study of their material did not aid in solving the problem whether myelopoiesis takes its origin in the spleen and liver or originates from primitive cells that are carried to these organs by the blood stream—that is, by colonization.

For extramedullary myelopoiesis in man even the careful analysis of numerous cases has not yielded a final solution, and Boyd⁴⁸ wrote in his textbook that there is no more histological evidence for the one view than for the other. It seems that colonization is easier to prove than local origin. The high frequency in bone marrow and in blood, the small perivascular foci in liver, spleen and lymph nodes and the histological picture of numerous immature blood cells migrating through vascular walls make colonization seem probable. It has to be admitted that the finding of blood cells in the vascular wall does not prove in which direction they migrate, whether from the lumen towards the perivascular tissue or in the opposite direction. The same doubt can be raised against sweeping conclusions based on higher concentrations of the immature blood cells in the vessels of the organs which harbor the extramedullary foci (observations by Lang,²³ Barnes and Sisman,⁴⁶ Askanazy,⁴⁹ Herzog⁵⁰). The cells may accumulate in these vessels before settling in the tissues, or they may have entered the vessels after originating in the local foci. If they are found in a typically restricted part of the vascular wall only—Jaffé,⁵¹ for instance, described subendothelial localization in the splenic vessels of leukemic patients—transmigration has to be dropped and local origin from this part of the vascular wall to be considered. The successful transmission of leukemia in mice by inoculation of a few cells or even of one single immature leukemic cell, as carried out by Furth and Kahn,⁵² supports the classification of leukemia as a special type of malignancy and the interpretation of colonization as a special type of metastasis. Here, too, a different possibility of interpretation must not be omitted. Even if leukemia could be definitely established as analogous with malignant neoplasms, it may follow the mechanism of some carcinomas of the skin or of the liver and originate multicentrically.

Local origin is in general more difficult to prove. Definite evidence could be found in a case with negative bone marrow and blood findings combined with myelopoietic foci in liver and spleen. Such a case has not yet been observed, and one may never be observed. It is not likely that the agent which provokes a myelopoietic metaplastic reaction in spleen and liver finds no response in the physiologically myelopoietic bone marrow. Positive findings in the bone marrow, liver and spleen, combined with negative findings in the blood as in aleukemic myelosis, make local origin highly probable, if we can rule out an aleukemic phase of an ordinary leukemia. In most cases of aleukemic myelosis a few immature blood cells can be found in the circulation, and observations in malignant neoplasia prove that very few floating cells are sufficient to give origin to extensive metastases or colonies. The distribution and frequency of the immature blood cells may increase the probability of colonization in single cases. Henschen⁵³ observed a man of 55 years with spindle cell sarcoma of the thyroid combined with eosinophil leukemia. Roessle, who did the autopsy and analyzed the microscopical findings, considered that the local accumulation of the eosinophile cells, particularly around the necrotic sarcoma deposits, had not only hematogenic but also local origin. He based his conclusion on the distribution of the infiltrating cells, which could not be explained fully by hematogenic invasion, although the patient had 79 per cent eosinophils among 312,000 white blood cells per cmm.

The great difficulty in proving local origin lies in the fact that we can discover the heterotopic myeloid foci of the liver and spleen only at autopsy. At the time when the leukemic patient dies, he generally has reached a progressive phase of his disease with highly positive blood findings which do not permit ruling out colonization definitely. After unsuccessful examination of numerous cases of myelosis, I finally found a case which offers more favorable conditions for proving local origin by presenting not only far-reaching quantitative but also qualitative differences between the immature blood elements of the hepatic and splenic foci and of the blood. There is one more way, as already mentioned, to prove the local origin of extramedullary myeloid foci. It can be accomplished if we can prove the development of the immature blood cells from sessile elements by demonstrating a transitional phase of this transformation *in situ*; that is, cells in the location of sessile elements, which still retain the shape and relations of the sessile cells, but with the nucleus and the protoplasm already showing the characters of immature blood cells. This way is not readily accessible for cells within the interstitial parenchyma, but is available for

endothelial cells, thanks to their characteristic and significant shape and location. Observations of this type have proved the transformation of Kupffer cells into monocytes or into megakaryocytes. The case I observed presented a local transformation of endothelial elements into eosinophile granulocytes.

REPORT OF CASE

The patient, a colored woman, 46 years of age, stated that she was well until 3 months before when she began to have digestive disturbance characterized by belching, epigastric fullness, nausea and frequent vomiting. About the same time she had noticed weakness. These symptoms progressed until at the time of examination she could not do her own housework and vomited at least once a day. About 3 weeks before she had noticed abdominal swelling and pain, which was constant, dull, and located in the left upper quadrant. During the preceding 3 days she had vomited practically after every meal.

Physical examination showed the temperature to be 101.8° F.; pulse, 114 per minute; respiration, 28 per minute; blood pressure, 140/90 mm. Hg. The heart and lungs were essentially negative. The abdomen was distended three fingersbreadth above the xiphoid-pubic line. The spleen was enlarged to fill the entire left abdomen and extended into the pelvis. The liver was two fingersbreadth below the right costal margin. In the right side of the pelvis, a rounded, grapefruit-sized mass was palpable. There was tenderness in the left upper quadrant.

Upon *vaginal examination* there was found a firm mass connected to the cervix and extending into both adnexal regions and anteriorly above the symphysis.

The *laboratory findings* were: Hemoglobin, 35 to 36%; red blood cells decreasing from 2,380,000 to 1,970,000; white blood cells decreasing from 196,800 to 141,200; lymphocytes, 2%; myeloblasts, 52%; neutrophile metamyelocytes, 5%; neutrophile myelocytes, 8%; polymorphonuclears, 30%; microcytosis, 2 plus. The bone marrow obtained by sternal puncture showed: 46.6% myeloblasts, 4.6% neutrophile promyelocytes, 1.4% neutrophile myelocytes, 2.2% neutrophile metamyelocytes, 3.4% eosinophile metamyelocytes, 6.2% neutrophile bandforms, 1.8% eosinophile bandforms, 4.2% polymorphonuclear neutrophils, 0.2% polymorphonuclear eosinophils, 22.0% basophils, 4.6% lymphocytes, 0.2% monocytes, 0.2% histiocytes, 0.4% irritation lymphocytes, and 2% normoblasts. The bone marrow was extremely hyperplastic. Maturation of the white cells showed a marked shift to the left, 46% of the cells being myeloblasts. Red cell formation was markedly depressed. A Kahn test was negative. Nonprotein nitrogen in the blood serum was 42 mg. %; creatinin, 1.4 mg. %; uric acid, 6.5 mg. %. The patient received several blood transfusions and ran a continuous septic temperature. Gallop rhythm developed 3 days after admission and she died 1 month after admission.

The *diagnosis* was acute myelogenous leukemia in a phase of acute exacerbation.

Gross Examination

The body (163 cm., 68 Kg.) was that of a well developed, well nourished colored woman. There was moderate chronic pitting edema of both legs. The pupils were contracted, round and equal, the conjunctivae, lips and oral mucosa were pale, as were also the nailbeds. The abdomen was slightly above the level of the chest. There were numerous pale striae over both lower quadrants of the abdomen and over the anterior aspect of both legs.

On opening the *abdominal cavity* the liver was found to extend 20 cm. below the xiphoid process in the midline and 3.5 cm. below the costal margin in the right axillary line. The enormously enlarged spleen extended to the navel and touched the left part of the uterine fundus. The uterus was markedly enlarged, of firm consistency and rendered irregular by several intramural and subserous fibroids. The fundus extended to the navel.

The *heart* weighed 430 gm., and was moderately dilated. The myocardium was pale, brownish and rather soft. The wall of the left ventricle measured 14 mm.; of the right, 3 mm. Both ventricles were filled with firm, pale yellow blood clots and similar clots were found in the large vessels.

Both apices of the *lungs* were free. All lobes were crepitant and on section the surface was light pinkish gray and moist.

The tracheal and bronchial *lymph nodes* were of normal size, soft and slightly anthracotic.

The mucosa of the *esophagus* was pale gray and had low folds. Over the left side of the cardia extended a large ulcer, 2 to 3 mm. deep, measuring 21 by 40 mm. The edges were irregular and sharply demarcated, but not undermined, the smooth gray floor was covered with a thin coat of brownish black, soft hemorrhagic material. The lower pole of the ulcer was 4 mm. below the junction of the gastric and esophageal mucosa. (Fig. 4.)

The *spleen* weighed 2560 gm., the consistency was firm, the capsule slightly thickened, the color dark purple-red, mottled with irregularly shaped, whitish, partially confluent spots. On transverse section it was dark purple, red and brown, mottled with a few paler, grayish areas. In the subcapsular region a few triangular, blackish areas appeared, representing old hemorrhagic infarcts. Where the pale areas reached the surface, the latter was slightly depressed.

The *liver* weighed 3250 gm., being markedly enlarged and firm. The capsule was thin and smooth. The sectioned surface was light brown and distinctly marked by a light periphery and slightly darker center of each lobule and by slightly enlarged periportal fields.

The *kidneys* weighed 325 gm. together and were soft. The capsule stripped with ease leaving a smooth, pale gray surface with distinct markings. On transverse section the cortex measured 9 mm., the pyramids, 12 mm. Their markings were indistinct and both were very pale. The mucosa of the pelvis was smooth and white.

The *uterus* measured 22 by 14 by 7.5 cm., containing several firm, intramural and subserous fibroids, the largest of which had a diameter of 7 cm.

The *bone marrow of the femur* was moderately soft. In part, it was of increased consistency comparable to the consistency of young granulation tissue; in part, soft and gelatinous; the color throughout was light pinkish gray.

The *lymph nodes* were of normal size and consistency. No enlarged or indurated lymph nodes were found.

Microscopical Examination

Liver. In size and shape the hepatic lobules were normal and the lobular structure was distinct. The central veins were slightly dilated and so were, in general, the central parts of the sinusoids, with resulting compression of the central ends of the liver cell trabeculae. Whereas the liver cells in the peripheral part of the trabeculae presented vesicular pale-staining nuclei and protoplasm with distinct plastosomes, the compressed cells had darker staining homogenous protoplasm loaded with brown bile pigment granules. In many acini the para-central sinusoids were markedly dilated by immature white blood cells, mostly stem cells or myeloblasts with basophile protoplasm. This accumulation of myeloid elements formed small pools extending between the compressed ends of the trabeculae. The portal triads presented a dense infiltration of the stroma, particularly around the portal veins, with some stem cells and numerous myelocytes and metamyelocytes, the majority of which were eosinophils. (Figs. 5 and 6.) Some eosinophile cells were seen in an irregular distribution in the wall of the portal veins. In the immediate vicinity of the bile ducts the eosinophils were not as numerous. The lumina of the vessels in the periportal fields contained few red blood cells, numerous stem cells with basophile protoplasm, promyelocytes, and only a very few eosinophile granulocytes. Whereas the numerous eosinophile cells in the stroma of the periportal fields were mostly of the early immature type—promyelocytes or myelocytes with round or oval, vesicular, pale-staining nuclei—the very few intravascular and intracapillary eosinophils were of the more mature type, mostly metamyelocytes with darker staining, bean-shaped nuclei. Some were even polymorphonuclears, with bisegmented dark nuclei.

Special staining, particularly with Giemsa's stain, showed that many endothelial cells of the sinusoids, while still presenting the normal stellate appearance and typical localization at the Disse space, had developed groups of typical eosinophile granules in their protoplasm. (Figs. 7 and 8.) Some possessed granules only on one side of the nucleus, some on both sides; others had the entire protoplasm filled with the granules. Among the last group, cells were found in which

the spindle-shaped projections of the protoplasm were retracted. In consequence, these cells, the nuclei of which had become more or less round and vesicular, were oval or spherical. Some cells in this phase of transformation were still in touch with the wall at a part of their circumference, other were free-floating in the lumen of the sinusoids. By a series of pictures, the transformation of the sessile endothelial cells, some of which were typically stellate, into free-floating eosinophile promyelocytes could be followed. The transformation was carried through *in situ* and only afterwards were the cells detached. Other endothelial cells were enlarged and their swollen protoplasm contained remnants of phagocytosed red blood corpuscles and of phagocytosed granulocytes. A few only contained small clusters of clumped, faded eosinophile granules, representing the last residues of digested eosinophils. This picture is entirely different from the transforming endothelium and cannot be confused with it. Neither the smaller nor the larger branches of the hepatic vein showed eosinophile cells in or next to the wall. Since liver cell trabeculae are anchored in the walls of the hepatic veins, there is neither an adventitia nor a perivascular stroma present which could contain undifferentiated cells ready for transformation. (Fig. 9.)

Evidently, not only are the external layers of the wall different in the hepatic and portal veins, but the endothelial cells also have different biological activity. In some cases of hemolytic anemia, when the Kupffer cells are loaded with hematogenic iron, the endothelial cells which line the hepatic veins similarly are stuffed with iron pigment, thus proving that they assume reticulo-endothelial functions analogous to those of the Kupffer cells. The endothelium of the intima of the portal veins (not seen in the illustration) remains absolutely free from iron pigment.

Spleen. The microscopical structure of the spleen was indistinct, due to numerous small and large and frequently confluent infarcts of varying ages. The greater part of the parenchyma was thus destroyed. The well preserved remainder presented no partition into follicles and pulp but instead a homogenous tissue consisting mostly of stem cells, myeloblasts, and some myelocytes. Many of these cells had undergone necrosis, as was shown by the homogenous, almost black, partially fragmented, pyknotic nuclei. The trabeculae were thin and indistinct. The stroma contained numerous thin-walled vessels and capillaries. Sinuses were indistinct. The parenchyma presented small groups of eosinophile elements, mostly promyelocytes and myelocytes. Some of these cells had stellate protoplasm with spindle-shaped projections which contained few or no granules. Evidently these cells had

developed from reticular cells of the pulp. In the lumina of the blood vessels was the same abundance of stem cells as in the peripheral blood of other organs and only a very few eosinophile cells, mostly metamyelocytes.

Kidney. The renal glomeruli were normal. The tubular epithelium was high cuboidal with finely granular protoplasm and pale-staining nuclei, some of which had completely faded. There was no myelopoietic tissue. The blood vessels contained the same immature myeloid cells as elsewhere, relatively many more red blood cells and few eosinophils.

Bone Marrow. The bone marrow showed extensive fibrosis. Large areas were occupied by interwoven bundles of collagenous connective tissue interspersed with small clusters of myelogenous cells. The hematopoietic tissue was restricted to about one-third of the space. It consisted of the same immature elements as seen in the foci in the liver, similarly interspersed with a great number of eosinophile promyelocytes and myelocytes. In some areas, the eosinophile cells were increased to about 90 per cent of all cells. Among the fibroblasts in the transitional borderline zones between hematopoietic and fibrotic tissues, spindle-shaped cells with longitudinal vesicular nuclei could be seen which contained eosinophile granules singly and in groups in the protoplasm. The fact that single collagenous fibers crossed the protoplasm of these cells or developed from it proved that we were not dealing with eosinophile granulocytes squeezed and flattened between neighboring fibroblasts or fibrocytes, but with cells which originate from undifferentiated mesenchymatous elements which, instead of developing normally into fibroblasts or into granulocytes, were undergoing a type of mixed or hybrid differentiation. They presented the form and the function of producing interstitial fibers like fibroblasts and granulation like that of eosinophile myelocytes. Transitional pictures made it likely that in the course of further development these cells lose their granulation and join the field of the fibrocytes.

Esophagus. The ulcer of the esophagus showed an almost vertical break in the stratified epithelium. The floor of the ulcer extended 3 to 4 mm. beneath the surface of the epithelium. It was formed by a 0.5 mm. layer of necrotic tissue, which still revealed a dense infiltration with immature white blood cells. This infiltration extended to the muscularis propria and split into small groups which could be found in the septa between the bundles of the circular layer. The cells were of the same types as were found in other organs, with no eosinophils among them. The blood vessels contained, as in the kidney, a high percentage of red blood cells with immature granulocytes and only very few mature eosinophils.

DISCUSSION

Eosinophil leukemia is not frequent compared to neutrophil leukemia. It seems that the frequency corresponds approximately to the physiological relation of eosinophile to neutrophile granulocytes, *i.e.*, about as 1 is to 75. The older literature can be found among the 2000 references which Schwarz⁵⁴ abstracted in his almost complete review on eosinophilia published in 1914. The more recent observations are critically reviewed by Forkner,⁵⁵ Ringoen⁷ and Richter.⁵⁶ Forkner analyzed several cases, among them five cases of acute eosinophil leukemia. No definite etiological factor can be given why these cases developed eosinophil and not, as do the great majority, neutrophil leukemia. Constitutional hereditary variations may be considered, but the fact that in some cases changes from neutrophil into eosinophil leukemia and vice versa have been observed, means that not too much emphasis should be placed on constitutional factors.

For local eosinophilia, split proteins, the structure of which is still unknown, have been considered as the incentive by Fiessinger⁵⁷ and others. Henschen⁵³ suggested a good practical classification of the various types of eosinophilia. He distinguished eosinophil chronic myelosis from zoogenic (parasitogenic), anaphylactic, compensatory, neurogenic (parasymphathogenic) and constitutional eosinophilia and tried to find a common mechanism for all by explaining them as reaction against still unknown, endogenous or exogenous proteins. His endeavors were contradicted by his collaborator Roessle who, in the discussion, emphasized that it had been impossible to find any common causal factor for the various types of eosinophilia.

Fibrosis of the bone marrow or myelofibrosis has been observed in some cases of leukemia. Reviews have been published by Wolf,⁵⁸ and by Mettier and Rusk.⁵⁹ The combination with polycythemia has been discussed by Hirsch.⁶⁰ The evaluation of some observations and statistics is complicated by the confusion of the terms osteosclerosis and myelofibrosis which signify entirely different changes. Osteosclerosis means concentric increase of the compact layer of the long bones at the expense of the spongiosa, by transformation of parts of the spongiosa into compacta through filling of the interstices between the bone trabecula with solid bone substance. Myelofibrosis means the degenerative replacing of the bone marrow by nonspecific fibrous tissue, analogous to the degenerative replacement by fat tissue in anemia. Osteosclerosis frequently is followed by myelofibrosis; primary myelofibrosis in general is not followed by osteosclerosis. In my case we have to deal with a pure myelofibrosis without evidence of osteosclerosis. The undifferentiated stem cells of the bone marrow which give

origin to red and white blood corpuscles probably are not the "youngest" elements of this tissue. In the reticulum of this mesenchyma, evidently cells are left which represent a still earlier embryonic and less differentiated or specialized phase, cells which may produce blood elements, endothelial elements, or unspecific fibrous tissue. Conditions are similar in the spleen, where, as Klemperer⁶¹ has suggested, mesenchymatous elements are present endowed with the capacity to form blood, interstitial, endothelial, and reticular cells. The differentiation of these cells, predominatingly or exclusively, into interstitial unspecific elements, causes fibrosis of the spleen as in splenomegaly with hepatic cirrhosis. In my case we see that these elements still produce myeloid and erythropoietic stem cells, but to a great extent they change into fibroblasts, which form nonspecific and even hyaline degenerating connective tissue, thus restricting the hematopoietic tissue.

Ulcers of the intestines developing from leukemic infiltrations have been observed in many cases. One of the earliest reports on leukemia, a case analyzed by Virchow⁶² in *Die krankhaften Geschwülste*, includes the description of leukemic ulcers in the upper ileum. Later, leukemic intestinal ulcerations developing usually from leukemic infiltrations have been described by Eichhorst⁶³ (case with fatal hemorrhage), Schultze,⁶⁴ Herxheimer,⁶⁵ Askanazy,⁴⁹ Wells and Maver⁶⁶ (who coined the term pseudoleukemia gastrointestinalis for diffuse and nodular gastrointestinal leukemic infiltration with secondary ulceration) and v. Müllern and Grossmann.⁶⁷ Boikan⁶⁸ published in 1931 a review of the literature with some cases of his own. Following the conception of Jaffé,⁶⁹ he concluded from the similarity of the intestinal ulcerations in true leukemia and in aleukemic leukemia that these two diseases are identical. Recently Jones⁷⁰ described two cases, one with perforation of a leukemic ulcer at the ileocecal junction. Ebstein,⁷¹ Mager⁷² and Barnick⁷³ have observed analogous ulcerative leukemic changes in the pharynx and larynx, and Askanazy⁴⁹ described the histological picture of corresponding changes of the gingiva. An ulcer of the esophagus has not yet been described, as far as I could check the literature.

This case is one of chronic myelogenous leukemia, evidently before the outbreak of an eosinophilic phase. The immature eosinophils are already in the tissues but not yet in the circulation in any considerable number. For the eosinophils, the aleukemic stage is just on the verge of changing into a leukemic phase. The production of the eosinophils in the bone marrow is not different from that found in cases of eosinophil leukemia. In the liver they develop from two sources: from the

endothelial cells of the sinusoids and from elements in the adventitia and in the stroma next to the vascular wall. The transformation of the sinus endothelium, which can be proved by the presence of transitional phases, gives full evidence of local origin of this type of granulocytes. For the eosinophils in the periportal fields, local origin is most probable. There is no evidence that the eosinophils which have developed from Kupffer cells immigrate in the liver parenchyma and settle about the periportal triads. Such wandering of primarily sessile vascular endothelial cells, marked by the phagocytosis of injected India ink, through the connective tissue to distant points has been observed by Herzog⁵⁰ in the frog's tongue. In my case there is no relation between the eosinophils of endothelial origin and those of perivascular origin, except that both enter the blood circulation. Colonization cannot be ruled out since there are eosinophils in the blood. But these circulating eosinophils on the average are of much higher maturity than the perivascular eosinophils in the liver triads, and a local rejuvenation of blood cells after colonization is most unlikely. Sections of the white, firm blood clot found in the left ventricle at autopsy gave a good impression of the smaller proportion of eosinophils of relatively higher maturity in the blood, as compared to those in the liver tissue. Whether in the periportal triads they developed from the adventitia and from elements of the surrounding stroma, or from the adventitia only and secondarily swarmed out in the neighborhood, cannot be decided by the analysis of the slides. If there was a simultaneous development in the adventitia and in the surrounding stroma, we have to suppose that the cellular elements in the stroma which are prone to this transformation are the more numerous the nearer to the adventitia the tissue is located. For the sinus endothelial cells, this case shows that in addition to the potency to form monocytes physiologically (Aschoff⁷⁴) and in monocytic leukemia (Jaffé³³), and megakaryocytes in atypical leukemia (Downey, Palmer and Powell³²), they also have the potency to change into eosinophile granulocytes.

SUMMARY

Two explanations have to be considered for the development of extramedullary myelopoiesis. The first is the assumption of local origin. This theory meets with greater probability when the organs in question are those normally concerned with hematopoiesis in fetal life, as the liver and spleen. In the anemias, the presence of erythropoietic tissue in these organs may be interpreted as a compensatory revival of an earlier function which physiologically is not carried on in extra-uterine life. A subgroup of local myelopoiesis which finds

good support in many animal experiments concerns the transformation of vascular endothelium (Dieckmann¹⁴), of perivascular adventitia cells (Bloom,⁴⁰ Herzog⁵⁰), or of undifferentiated mesenchymatous elements mostly located in the vicinity of blood vessels (Jaffé,^{69, 75} Lang^{10, 23}) into undifferentiated lymphoid cells or hemocytoblasts, after irritation or stimulation by injection of dead bacteria, as *Bacillus proteus* or *B. coli*; of poisons, as saprotoxin, or pyrogallol; or after inoculation with transplantable tumors. The second theory traces the extramedullary foci of myeloid tissue back to hematogenous immigration of immature bone marrow cells which enter the circulation. This process, which is analogous to the metastasizing of malignant tumor cells by the blood stream, has been called by some authors *colonization*, a term coined instead of using metastasis in order to avoid an anticipated analogy of leukemic blood cells with malignant neoplastic cells.

For the extramedullary myelopoiesis in human leukemia, the question, as Boyd⁴⁸ points out, has not yet been decided, whether we have to deal with autochthonous local origin or with colonization. A high percentage of cells in the blood and in the bone marrow, small perivascular foci, and the histological picture of numerous immature bone marrow elements in process of migration through vascular walls, make colonization probable. Local origin is much more difficult to prove. Two ways are open. The first of these is to find evidence of local transformation of mesenchymatous cells, particularly of cells of the reticulo-endothelial system, into immature blood cells, that is, metaplasia *in situ*, by which these cells change into blood cells. Such observations are in general very difficult when reticular elements are concerned, but easy and obvious as far as endothelial cells are concerned, which by shape and localization are well characterized and easily recognizable. Bloom¹ succeeded in animal experiments in provoking transformation of Kupffer cells into megakaryocytes. The analogous change was observed in a case of atypical leukemia by Downey, Palmer and Powell.³² Jaffé³³ presented in one of his conferences a case of monocytic leukemia with positive oxidase reaction of the Kupffer cells. I have found similar changes in a case of chronic myelogenous leukemia in an acute monocytic phase. The liver showed the same change of the Kupffer cells, which, after transformation into round or oval cells, entered the circulation as large oxidase-positive monocytes.

The second way is to find a case which shows an abundance of the immature blood elements in the bone marrow and in well developed extramedullary foci, with the circulating blood free from such cells. Quantitative differences between the incidence of immature blood cells

in the blood and in the tissue do not prove much, since even a few intravascular cells may give origin to extensive metastases as we observe in malignant tumors. Only qualitative differences are convincing, that is, absence of the immature elements in the blood, which are present in the tissues. Application of this criterion meets with the difficulty that generally the blood is highly abnormal in the phase in which a patient with leukemia comes to autopsy. Only if, by chance, a patient dies at a time when the tissues contain great numbers of immature blood elements which not only are absent in the circulation at the time of autopsy but, as proved by the history, never were present (to avoid confusion with aleukemic leukemia or an aleukemic phase of a common leukemia), can it be applied. Only then can local origin of the extramedullary foci be established. After unsuccessful examination of numerous cases of acute and chronic lymphatic and myelogenous leukemia, a case of a colored woman, 46 years old, with chronic myelogenous leukemia was found, who expired at a time when the neutrophil leukemia was on the verge of changing into an eosinophil leukemia. Whereas the bone marrow and the liver at autopsy contained huge numbers of eosinophile promyelocytes and myelocytes, only the last blood examination a short time before death showed a low percentage of eosinophile metamyelocytes and polymorphonuclears. Slides of the liver gave evidence of a gradual transformation of Kupffer cells into eosinophile myelocytes by developing eosinophile granules and retraction of the stellate protoplasmic projections. After this metaplastic change, the cells entered the blood of the sinusoids as eosinophile granulocytes.

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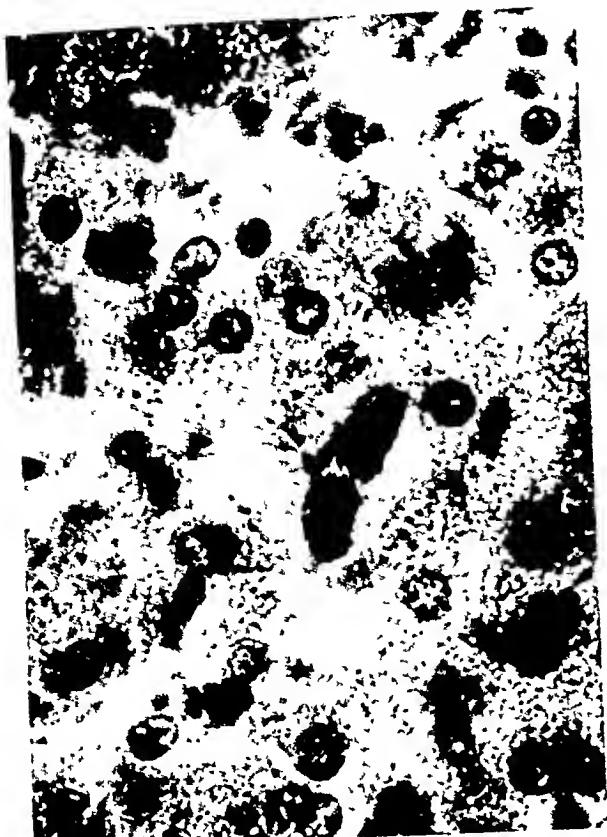
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[*Illustrations follow*]

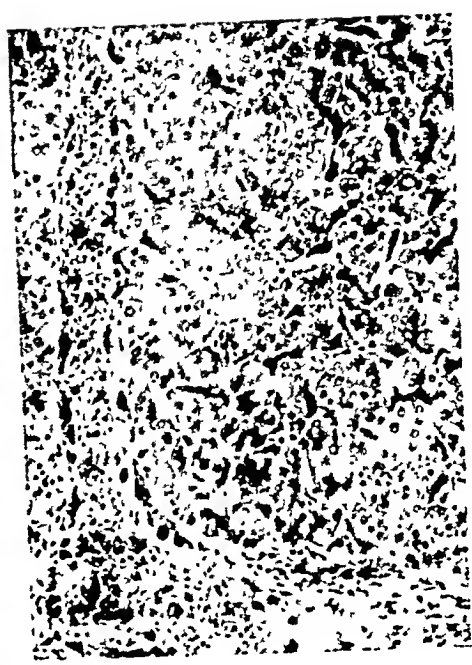
DESCRIPTION OF PLATES

PLATE 99

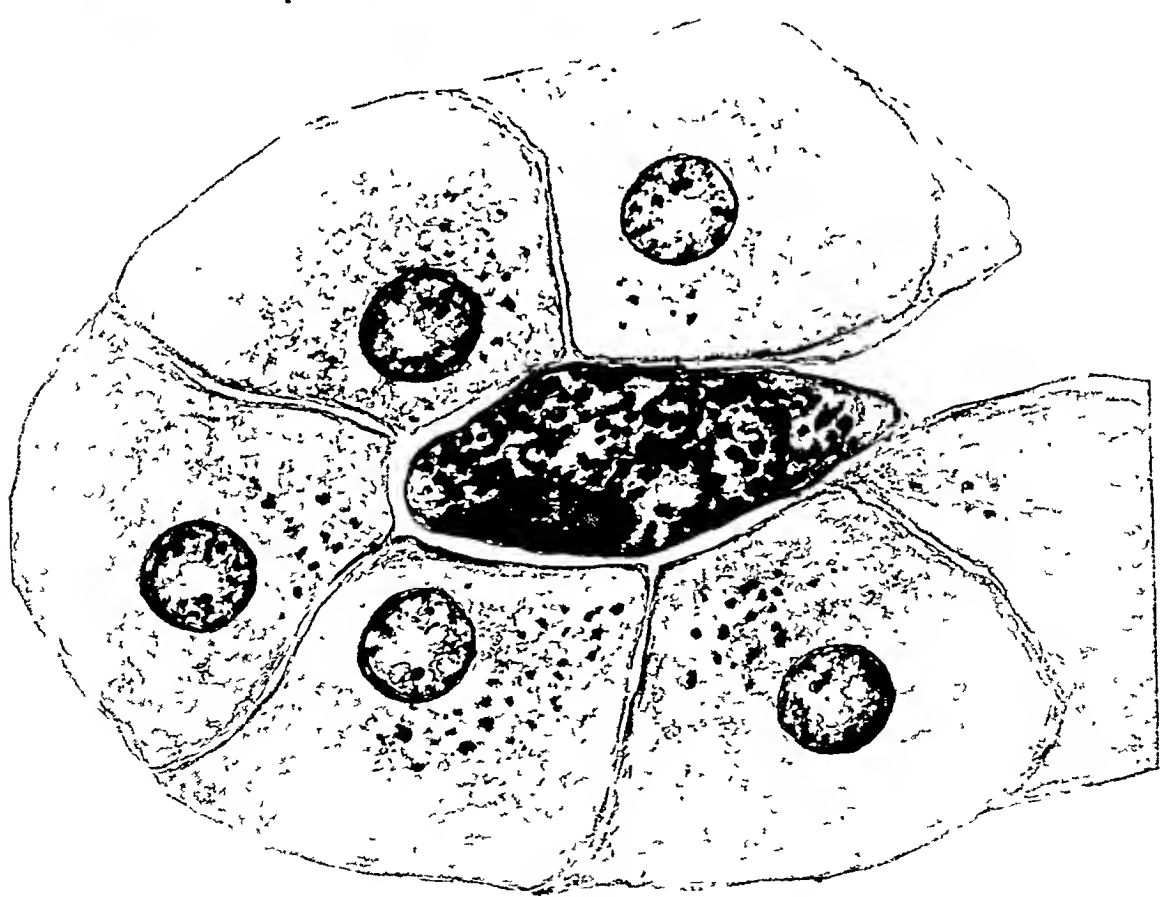
- FIG. 1. From a patient with monocytic leukemia. Liver with oxidase-positive granules in the Kupffer cells. A spindle-shaped Kupffer cell can be seen in the center, the protoplasm of which is stuffed with oxidase-positive granules. The lumen of the sinusoid at the top contains several oxidase-positive mononuclear cells. Oxidase reaction of Goodpasture. $\times 480$.
- FIG. 2. From the same patient as Figure 1. Kupffer cell with numerous oxidase-positive granules, surrounded by five liver cells in the corner of a sinusoid.
- FIG. 3. Periportal cirrhosis and hemochromatosis in a white man, 59 years of age. Specimen obtained from the liver for biopsy. The Kupffer cells, particularly in the upper right portion of the field, are full of iron pigment, and so, also, are the endothelial cells of the small branch of the hepatic vein at the left. Turnbull's-blue stain. $\times 50$.



1



3



2

Schiller

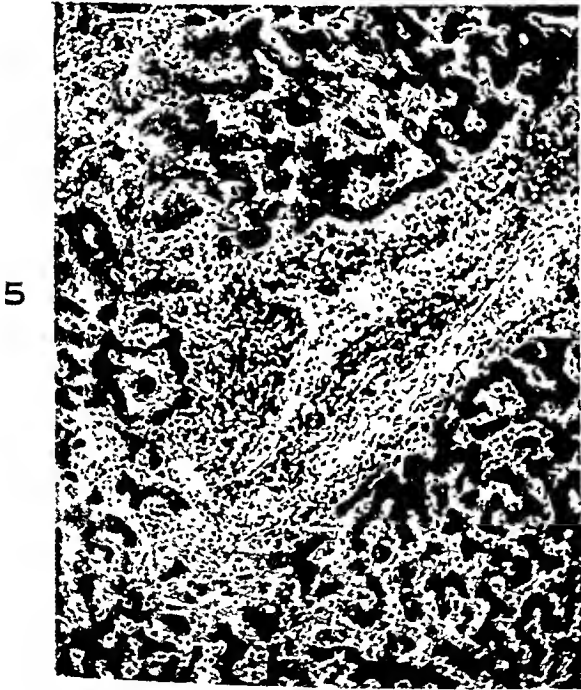
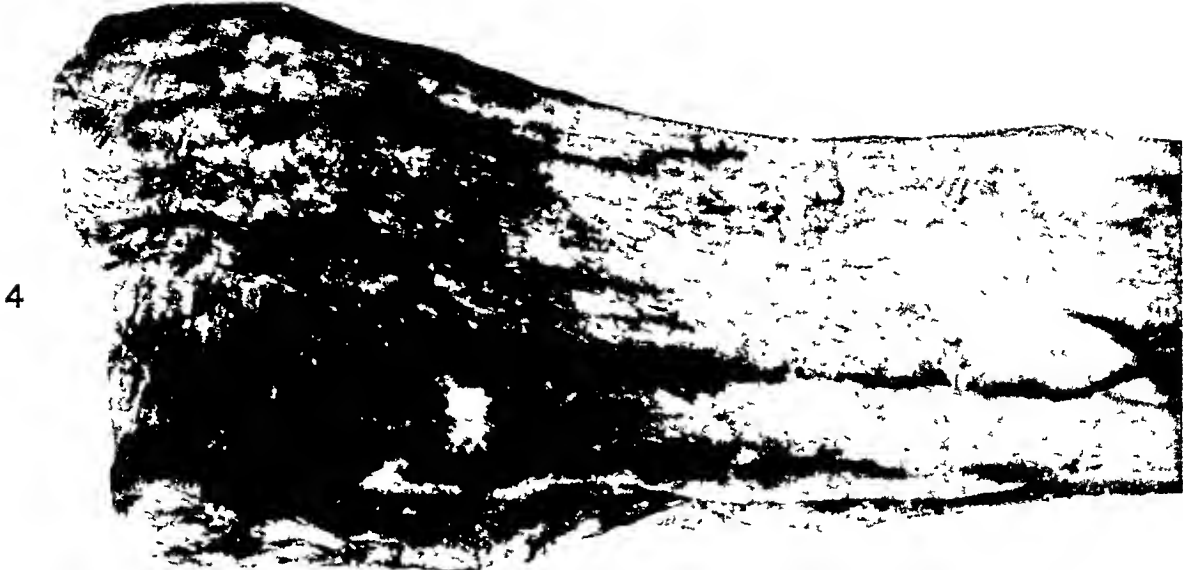
Local Myelopoiesis in Myeloid Leukemia

PLATE 100

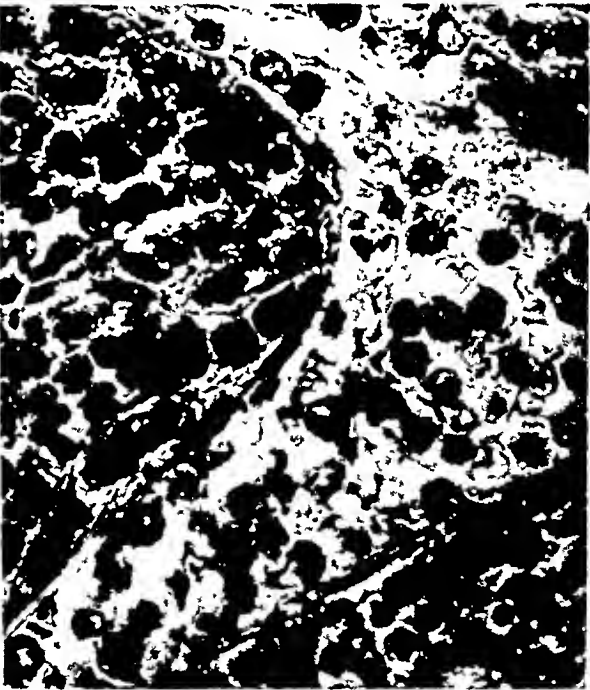
FIG. 4. From a patient with myelogenous eosinophil leukemia. Ulcer of the esophagus at the cardia.

FIG. 5. Myelogenous eosinophil leukemia. The stroma around a branch of the portal vein is infiltrated with eosinophile myelocytes. The lumen of the vein contains only a very few mature eosinophils. $\times 70$.

FIG. 6. The bifurcation of the vein shown in Figure 5. $\times 355$.



Schiller



Local Myelopoiesis in Myeloid Leukemia

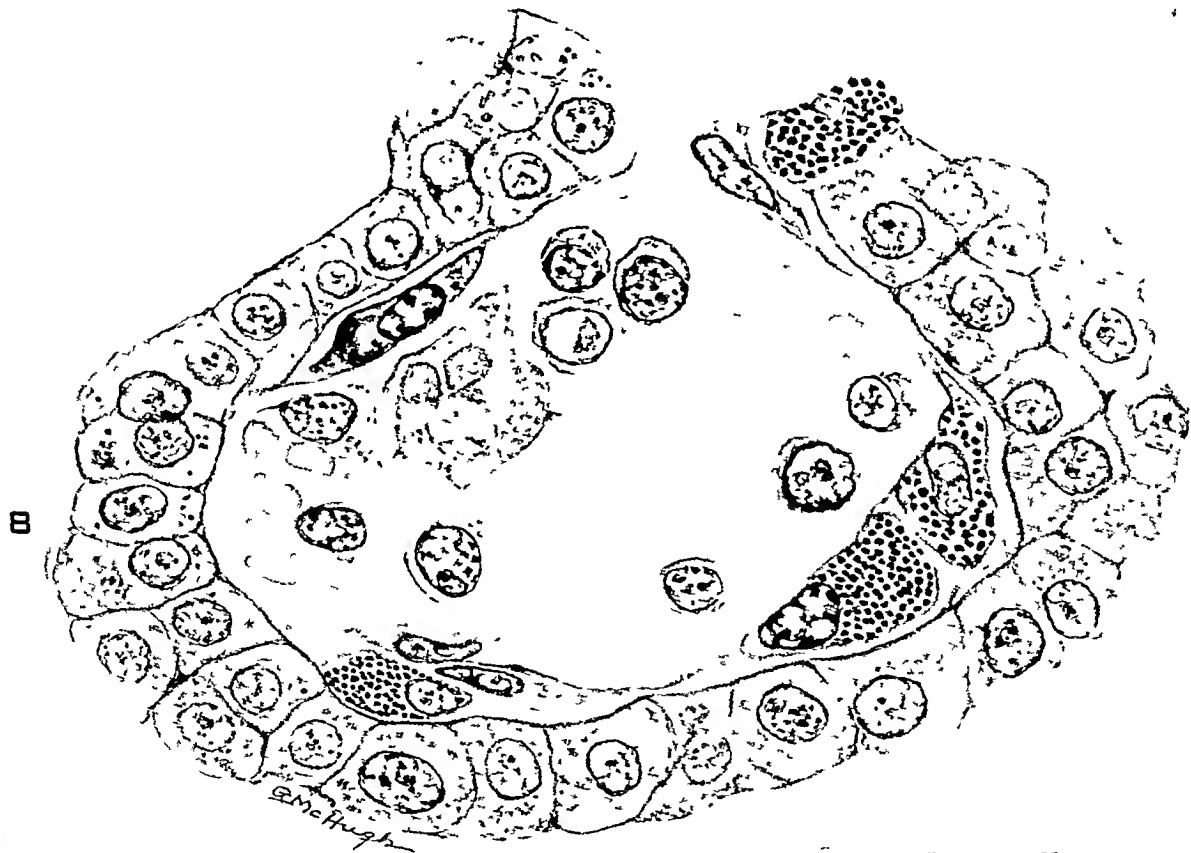
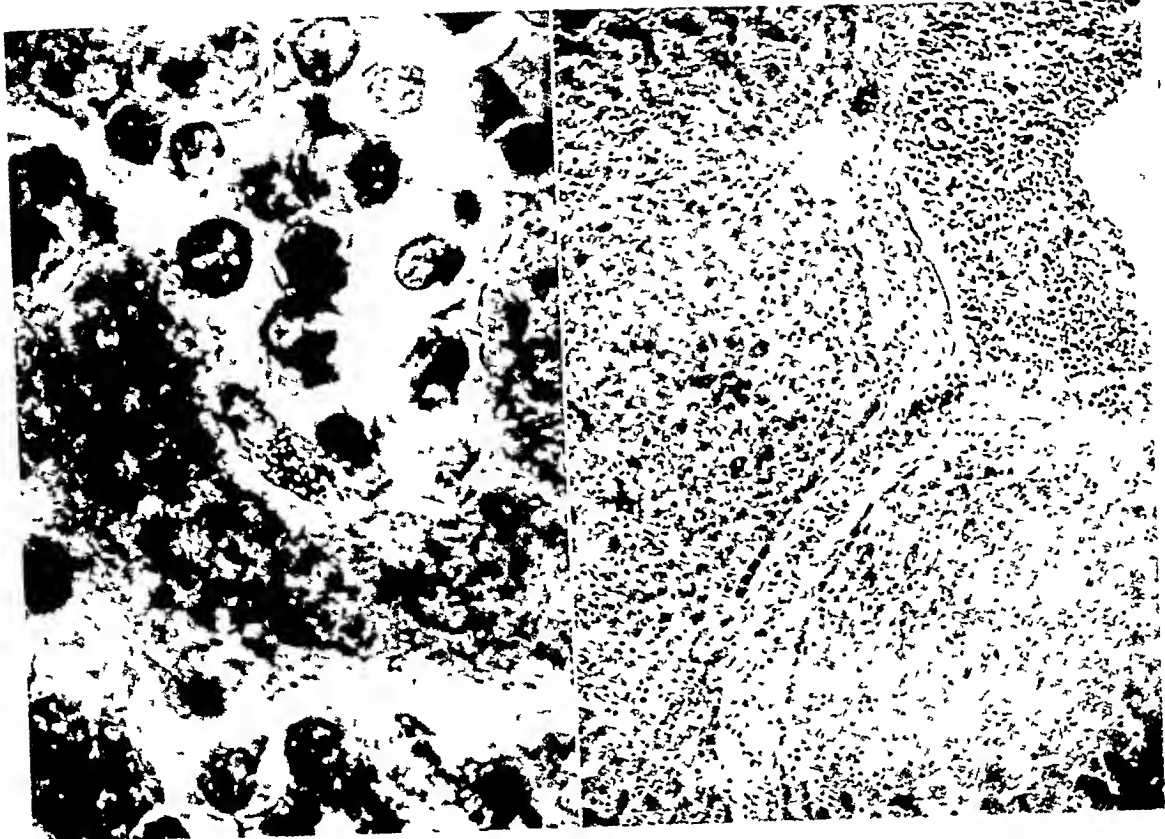
PLATE 101

Figures 7, 8 and 9 were made from sections from the same case of myelogenous eosinophil leukemia as the preceding three illustrations.

FIG. 7. One Kupffer cell in the sinusoid is loaded with eosinophile granules. $\times 800$.

FIG. 8. Three Kupffer cells have developed eosinophile granules. There is swelling of the protoplasm as a result of the granulation. In the upper part of the lumen there is a large, square, free-floating phagocyte, the protoplasm of which contains palely staining remnants of partially digested red blood cells.

FIG. 9. A small branch of the hepatic vein at the point of union with a larger trunk. There are no eosinophils along the walls. $\times 80$.



Schiller

Local Myelopoiesis in Myeloid Leukemia

THE EFFECTS OF PARATHYROID HORMONE AND CALCIUM GLUCONATE ON THE SKELETAL TISSUES OF MICE *

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The results of experiments on the effect of parathyroid hormone on the skeleton are not in full agreement. Cartilaginous overgrowth, stunting, or lack of any effect on body growth has been reported. On the other hand, increased resorption as well as increased formation of bone has been noted. The discrepancies in the findings seem to be due to several factors, such as species and age of the animal, the dose of the hormone, and the calcium and phosphorus intake. It is still being discussed¹ whether the temporary rise in the serum calcium is a primary effect of parathyroid hormone, or whether it is secondary due to an increased phosphorus excretion.² Administration of calcium gluconate likewise raises the serum calcium, although to a lesser degree and for a shorter period than parathyroid hormone.³⁻⁵

In continuation of former investigations on the effects of hormones on cartilage and bone,⁶ we thought it of interest to study whether increased serum calcium on the one hand, and parathyroid hormone on the other, affect skeletal development and ageing.

MATERIAL AND METHODS

Seventy-six male mice of the closely inbred strains C57 and CBA were used. The animals were kept on a standard diet of Purina chow with water available at all times. One group consisted of 38 growing mice, 4 to 9 weeks old at the beginning of the experiment. A second group consisted of 38 adult mice 12 to 14 months old at the beginning of the treatment. Further details are given in Table I.

The following three series of experiments were carried out:

Series I (28 Mice). Fourteen growing and 14 adult animals were injected intraperitoneally with parathyroid hormone.† Two animals from each group were treated daily for 1, 2, or 4 consecutive days, and 2 animals were injected three times weekly for 1, 2, 4, or 8 weeks.

* These experiments were conducted in the Laboratory of Research Pathology, Washington University, School of Medicine, St. Louis, Mo.

The investigation was carried out by the aid of grants from the International Cancer Research Foundation, from the Jane Coffin Childs Memorial Fund for Medical Research, and from the Committee on Research in Endocrinology of the National Research Council, given to Dr. Leo Loeb; and by the Albion O. Bernstein Fellowship in Pathology, New York University, College of Medicine.

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† Parathormone, Eli Lilly and Co., Indianapolis, Ind.

TABLE I
Mean Initial and Final Weights and Deviations from These Means in Pairs of Treated Mice

	Growing mice				Adult mice		
	Duration of experiment	Age at beginning of experiment	Initial weights gm.	Final weights gm.	Age at beginning of experiment	Initial weights gm.	Final weights gm.
A. Mice receiving parathyroid hormone	1 day	6 weeks	15.4 ± 1.0	13.0 ± 0.2	14 months	21.5 ± 0.5	20.5 ± 0.5
	2 days	6 weeks	16.1 ± 1.0	13.9 ± 0.3	14 months	23.2 ± 0.2	19.8 ± 0.0
	4 days	6½ weeks	18.0 ± 2.0	19.4 ± 2.3	13 months	28.1 ± 1.4	29.5 ± 2.0
	1 week	4½ weeks	10.9 ± 0.5	15.0 ± 0.6	12 months	27.3 ± 0.9	28.0 ± 0.2
	2 weeks	5½ weeks	12.3 ± 1.4	22.2 ± 0.2	14 months	23.8 ± 1.7	27.0 ± 2.0
	4 weeks	6 weeks	17.0 ± 0.4	23.5 ± 1.6	12 months	27.9 ± 1.2	27.9 ± 1.2
	8 weeks	5½ weeks	13.2 ± 0.6	23.9 ± 1.0	12 months	28.5 ± 0.7	28.5 ± 0.7
B. Mice receiving calcium gluconate	1 day	4 weeks	9.3 ± 0.5	9.5 ± 0.5	13 months	27.7 ± 2.3	28.0 ± 1.5
	2 days	5 weeks	12.2 ± 0.2	11.4 ± 0.2	13 months	26.4 ± 0.6	25.5 ± 0.2
	4 days	4 weeks	10.3 ± 0.0	9.9 ± 0.0	12 months	19.6 ± 0.0	22.8 ± 0.0
	1 week	6½ weeks	16.5 ± 0.8	16.4 ± 0.7	14 months	26.9 ± 4.0	25.1 ± 5.2
	2 weeks	5 weeks	11.9 ± 0.0	16.6 ± 0.0	12 months	26.8 ± 4.5	28.2 ± 3.5
	4 weeks	8 weeks	20.5 ± 0.0	24.7 ± 0.0	12 months	30.3 ± 0.4	30.2 ± 0.4
	8 weeks	5½ weeks	12.8 ± 1.0	24.7 ± 1.5	12 months	26.4 ± 0.0	28.8 ± 0.3
C. Mice receiving parathyroid hormone and calcium gluconate	1 week	4 weeks	10.1 ± 0.0	10.4 ± 0.3	12 months	26.3 ± 2.5	25.7 ± 3.0
	2 weeks	5½ weeks	14.7 ± 0.2	20.5 ± 0.3	12 months	23.6 ± 0.0	25.2 ± 0.0
	4 weeks	9 weeks	22.4 ± 0.2	23.5 ± 0.7	13 months	28.5 ± 2.1	25.8 ± 3.6
	8 weeks	6 weeks	15.0 ± 1.1	25.5 ± 1.3	12 months	25.2 ± 1.3	29.2 ± 1.0

Growing animals received 4 units, adult animals 7 units of the hormone at each injection.

Series II (28 Mice). Fourteen growing and 14 adult animals received intraperitoneal injections of 0.25 cc. of a 10 per cent solution of calcium gluconate.* Two animals from each group were treated daily for 1, 2, or 4 consecutive days, and 2 animals from each group were injected three times weekly for 1, 2, 4, or 8 weeks.

Series III (20 Mice). Ten growing and 10 adult animals received a combined administration of parathyroid hormone and calcium gluconate for 4 consecutive days, 1, 2, or 8 weeks. The dose administered corresponded to that given in series I and II.

As far as possible, litter mates were used. Additional mice of the same strains, sex and age served as normal controls. Sections of tibia and femur were prepared as described in previous investigations.⁷ The epiphyseal disk at the upper tibia was selected as the area where any changes could be best studied.

A. EFFECTS OF PARATHYROID HORMONE

I. Growing Animals

(a) Gross Observations

The treated animals lost 10 to 20 per cent of their initial weights after the first two injections (Table I, section A). The initial weights were regained after the fourth injection. Subsequently, the weight increase was rapid and surpassed that of noninjected animals.⁸ After 1 and 2 months of treatment, the weights equalled those of noninjected mice.

(b) Histologic Examination

(1) *Epiphyseal Disk.* After one injection of 4 units of the hormone, the zone of endochondral ossification showed regular structure in the cartilaginous cell columns. The cartilage rows were separated from one another by thin strips of chondromucoid ground substance. The total number of cells in a single cartilage row was 10, as is normal for this age. However, the various cells were larger than the corresponding cells in untreated animals. The nonoriented cartilage cells were rounded off; their conversion into columnar cartilage cells was accelerated. The latter cells exhibited marked proliferation by mitosis. The onset of hypertrophic changes took place farther proximally than in control mice. The replacement of the hypertrophic cartilage cells by bone was increased (Figs. 1 and 2).

After two injections, the columnar cells in a single column had decreased to 6 or 7 (instead of 10), and the hypertrophic cells to 2 or 3

* Abbott Laboratories, North Chicago, Ill.

(instead of 4, as is normal). Simultaneously, more calcium than usual had been deposited in cartilage cells and matrix. The nonoriented cartilage cells had assumed a resting condition. The proliferation, and hypertrophy of the cartilage were decreased. The ossification of the densely calcified cartilage was more accentuated than after one injection.

After 4 days' treatment, the growth zone was heavily calcified (Fig. 3). The proliferation of the columnar cartilage was greatly diminished or at a standstill, but the replacement of cartilage by bone was increased.

At later stages, the cartilage underwent marked regression. Degenerated cartilage rows appeared in the epiphyseal plate of mice 7 or 8 weeks old injected for 2 weeks. These changes were not found in healthy untreated animals of this strain before the end of the fourth month of life.

After 1 or 2 months of treatment, the epiphyseal disk was distinctly narrowed. Numerous thick, amorphous, sometimes ossified plugs had replaced the degenerated cartilage rows. In addition, increased resorptive processes had begun to dissolve the amorphous or bony plugs, and perforations of the epiphyseal plate were noted (Fig. 4). Thus, in 10 weeks old mice of strain C57, injected for 4 weeks, skeletal development and ageing had reached a degree seen in healthy noninjected mice of this strain only toward the end of the first year of life.

(2) *Subepiphyseal Layer*. In untreated animals, the subchondral zone consists of a loose, vascular connective tissue, and of bony spicules separated from one another by cellular marrow. After one injection, osteoblasts proliferated mitotically and filled the intertrabecular spaces (Fig. 2). Farther distally, along the pre-existing bony spicules, the mitotic proliferation of osteoblasts was likewise stimulated (Fig. 5). These changes were further accentuated after two injections. The conversion of osteoblasts into osteocytes was increased; the trabeculae were thickened, elongated, more numerous, and connected with one another by bony links.

After 4 days of injections, the spicules were numerous and densely calcified, and a transverse osseous plate was being formed underneath the epiphyseal cartilage.

After 1 or 2 weeks, cellular and vascular resorption predominated over the formation of bone, and the excess bone present at the earlier stages disappeared. After 1 or 2 months of treatment, the metaphyseal spicules in the center had been dissolved, whereas at the periphery there were still numerous thickened trabeculae. The bone marrow had not undergone fibrotic change.

(3) *Joint*. During the first 2 days of treatment, the articular cartilage cells were hyperplastic and hypertrophic. Later, the hyperplasia ceased, and the increased hypertrophy was followed by intensified ossification. After 2 weeks of injections and later, the cartilaginous matrix had increased in amount and density.

(4) *Shaft*. Subsequent to the first, second and fourth injections, the compacta was thick and dense. The spindle cells at the inner and outer surfaces of the shaft proliferated mitotically. Many of these cells had been converted into large osteoblasts lying close together (Figs. 6 and 7). After continuation of the treatment for 1 or 2 weeks, however, the proportion between spindle cells and osteoblasts was changed in favor of the spindle cells. After 1 month of treatment, the inner surface of the shaft was again lined by a single layer of endosteal cells as is normal (Fig. 8).

II. Adult Animals

(a) Gross Observations

After administration of 7 units of the hormone for 1 or 2 days, the animals lost on the average 10 to 12 per cent weight (Table I, section A). Subsequently, there was a steady weight increase that sometimes surpassed that of control mice.

(b) Histologic Examination

(1) *Epiphyseal Disk*. In untreated old mice, the epiphyseal plate consists of calcified, hyalinized, or ossified cartilage, in some places perforated by bone marrow.

During the first 2 weeks of injections, the ossification of the calcified and hyalinized cartilage was farther advanced than in untreated animals. After 1 or 2 months of treatment, a large part of the strongly calcified cartilage was ossified.

(2) *Subepiphyseal Layer*. In untreated mice, the metaphyseal bone undergoes increasing resorption with advancing age. Following treatment from 1 to 4 days, the transverse osseous lamella underneath the epiphyseal cartilage was thin; in some areas, calcified cartilage was in direct contact with the bone marrow. Simultaneously, an increased number of osteoblasts had been formed underneath the thinned osseous plate and on the surface of such trabeculae as were present. At later stages of the experiment, even more bone had been laid down. Thus, transverse trabecular bony structures of considerable thickness appeared in the metaphysis (Figs. 9 and 10). There was no increase in the amount of connective tissue in the diaphyseal marrow.

(3) *Joint*. Administration of parathyroid hormone did not affect the

incidence and severity of the age changes taking place in the articular cartilage in normal old mice.⁹

(4) *Shaft*. No changes were observed under the influence of the hormone.

B. EFFECTS OF CALCIUM GLUCONATE

I. *Growing Animals*

(a) Gross Observations

The treated animals did not gain, or even lost weight, during the first week of the injections. Subsequently, the weights increased, and after 1 or 2 months they did not differ from those of the control animals (Table I, section B).

(b) Histologic Examination

(1) *Epiphyseal Disk*. After one injection, but more pronouncedly after two or four injections, the zone of endochondral ossification was narrower than usual. The cell count was 6 or 7 (normal 10) columnar, and 1 or 2 (normal 4) hypertrophic cells in a single cartilage row in animals 5 weeks old. The nonoriented cartilage cells were resting. The columnar cartilage cells were flatter than ordinarily; their proliferation was decreased, mitoses being scarce or lacking. The hypertrophic cartilage cells were also decreased in size and heavily calcified. The largest cells present had the size of large columnar cells of untreated mice.

After 1 or 2 weeks of treatment, the columnar cartilage was less atrophic and its proliferation was less inhibited than at the earlier stages. Larger hypertrophic cartilage cells were found, and their replacement by bone had made good progress. After 1 or 2 months of injections, the height of the growth zone did not differ from that of the untreated animals. However, the structural age of the epiphyseal cartilage was advanced over the normal. Hyalinization and calcification were marked, and amorphous plugs of disintegrated cartilage appeared in the epiphyseal disk at the upper tibia in mice 12 weeks old (Fig. 11). This condition is not observed in untreated healthy mice of this strain and age. The younger the animal at the beginning of the injections, the more readily it responded to the treatment.

(2) *Subepiphyseal Layer*. After 1, 2, or 4 injections, the subchondral zone was poorly vascularized. The bony spicules were well formed and strongly calcified; they consisted of compact osteocytes and dense ground substance. The mitotic proliferation of osteoblasts was not increased. After 1 or 2 weeks of treatment, the vascularization of the subepiphyseal zone had increased; intensified resorption had led to a shortening of the trabeculae which in their proximal parts were

connected by transverse osseous links. At later experimental stages, a bony lamella underneath the epiphyseal cartilage had formed in mice of strain C57 13 weeks old. In untreated animals of this strain, a corresponding condition is not seen before the age of 4 or 5 months.

(3) *Joint*. The articular cartilage showed no significant changes throughout the experiments.

(4) *Shaft*. During the first week of injections, the compacta was heavily calcified and denser than usual. With increasing duration of the experiment, the bony cortex assumed a normal firmness.

II. Adult Animals

(a) Gross Observations

No influence on the weights of the animals was noted (Table I, section B).

(b) Histologic Examination

(1) *Epiphyseal Disk*. At no experimental stage was there any evidence of an effect of calcium gluconate on the inactive cartilage cells.

(2) *Subepiphyseal Layer*. During the first 2 weeks of treatment, the transverse subchondral bony plate was thin. After 1 or 2 months, osteocytes in increased number were laid down along the transverse osseous plate.

(3) *Joint*. The incidence and severity of the age changes found in the articular cartilage did not differ from those noted in untreated animals.

(4) *Shaft*. No deviation from the normal condition could be detected.

C. EFFECTS OF COMBINED ADMINISTRATION OF PARATHYROID HORMONE AND CALCIUM GLUCONATE

I. Growing Animals

(a) Gross Observations

During the first week of treatment, the weights remained stationary. Subsequently, the animals gained weight steadily, and finally the weights equalled those of the controls (Table I, section C).

(b) Histologic Examination

(1) *Epiphyseal Disk*. After 4 days of treatment (8 units of parathyroid hormone and 0.5 cc. of calcium gluconate) the growth zone was narrowed.

The nonoriented cartilage cells were in a resting condition. Proliferation and hypertrophy of the columnar cartilage were decreased; calcification and ossification, however, were intensified as compared with the normal. After 1 week of injections (12 units of parathyroid hor-

mone and 0.75 cc. of calcium gluconate) the epiphyseal disk was narrower than at the earlier stage. In a single cartilage row 6 or 7 (instead of 10) columnar and 2 or 3 (instead of 4) hypertrophic cartilage cells were counted. Calcification and ossification of the cartilage were marked. Subsequently, regression and ossification of the cartilage had increased still further, and thick bony plugs had replaced cartilage rows. These changes did not make progress during the second month of treatment. Resorptive processes were less marked than after administration of parathyroid hormone only. In one 3 months' old mouse, advanced resorption had produced small perforations of the epiphyseal disk. In the three remaining mice, 12 to 15 weeks old, and injected for 1 or 2 months, the structural age of the epiphyseal cartilage was comparable to that of noninjected mice 6 to 8 months of age.

(2) *Subepiphyseal Layer*. After 4 days of treatment, numerous mitotically proliferating osteoblasts had produced an osteogenic tissue. The trabeculae were thicker than normal, and thicker than after injections with parathyroid hormone alone for the same time. After 1 week of injections, intense resorption had begun to remove the bony spicules in the central portions of the metaphysis; however, the deposition of bone in close approximation to the epiphyseal cartilage was still increased, and osseous links between the spicules appeared. With longer duration of the experiment, both formation and resorption of bone remained accentuated. The resorption of bone was less pronounced than after administration of parathyroid hormone, but more marked than after injections of calcium gluconate alone.

(3) *Joint*. During the early experimental stages, ossification of the articular cartilage was intensified; later, resorptive processes predominated. After 2 months of injections, the condition was not different from the normal.

(4) *Shaft*. During the first 2 weeks of treatment, the compacta was thicker and denser than usual. Later, intensified resorption had led to the usual appearance of the cortex.

II. Adult Animals

(a) Gross Observations

Some animals showed stationary weights, others lost weight up to 15 per cent, still others gained weight (Table I, section C).

(b) Histologic Examination

(1) *Epiphyseal Disk*. Throughout the experiments, remnants of markedly calcified, hyalinized, or ossified cartilage were present. The degree of perforations of the disk by bone marrow was not advanced over the normal.

(2) *Subepiphyseal Layer*. During the first 2 weeks of treatment, the subepiphyseal bony lamella was thin. After 1 or 2 months of injections, production of bone was prominent. Osseous tissue had been deposited in the corners between the metaphyseal part of the shaft and the heavily calcified remnants of epiphyseal cartilage. An irregular bony network appeared parallel to and underneath the pre-existing transverse osseous plate, and one or several thick bony bars had been laid down in the metaphysis (Fig. 12). This excessive bone formation had inhibited the normal progress of epiphyseo-diaphyseal union.

(3) *Joint*. The injections did not affect the incidence nor the severity of the arthropathic age changes found in untreated old mice.

(4) *Shaft*. The compacta was thin at the earlier stages of the experiments, as is normal for old mice. After 1 or 2 months of treatment, the cortex consisted of more and denser bone than ordinarily.

DISCUSSION

In immature mice, parathyroid hormone intensifies hypertrophy and ossification, but does not stimulate the proliferation of the epiphyseal cartilage. It increases the mitotic proliferation of osteoblasts and thus increases bone formation. Subsequently, increased regressive and resorptive processes cause a premature onset of perforations of the growth zones and a removal of the excessive amounts of bone. Parathyroid hormone thus promotes the ageing of the epiphyseal cartilage. In old mice, parathyroid hormone increases the calcification of the inactive epiphyseal cartilage and intensifies deposition of bone in the metaphysis.

In growing mice, calcium gluconate promotes temporarily calcification and ossification, but inhibits proliferation, hypertrophy and resorption of the epiphyseal cartilage. It increases the density of the bone, but does not stimulate the mitotic proliferation of osteoblasts. Subsequently, proliferation and hypertrophy of the cartilage are resumed, and regressive and resorptive processes are intensified as compared with the earlier stages. Calcium gluconate thus likewise accelerates skeletal ageing, but to a far less degree and for a shorter length of time than parathyroid hormone. In old mice, calcium gluconate does not affect the inactive epiphyseal cartilage. However, it decreases the resorption of bone. Consequently, in the metaphysis and in the shaft more bone is present than usual.

In growing mice the combined administration of both substances does not intensify the accelerated ageing of the epiphyseal cartilage caused by parathyroid hormone alone. However, the resorption of cartilage and bone is less marked than after injections of parathyroid

hormone only, and more bone is present than after administration of either substance alone. In old mice the combined treatment produces considerable amounts of new bone, particularly in the metaphysis.

Table II summarizes the histologic findings in a schematic way.

The ageing effect that parathyroid hormone exerted on the epiphyseal cartilage of growing mice can be correlated with the gross observations of stunting made in growing rats,^{10, 11} dogs¹² and axolotl¹³ under the influence of this hormone. Parathyroid hormone not only accelerates the ageing of the epiphyseal cartilage, but may also stimulate the formation of bone. The intensified calcification of the epiphyseal cartilage may by itself promote the production of bone. Moreover, the increased deposition of bone may be the consequence of the stimulation of the connective tissue cells. If, as in our mice, sufficient calcium is available, excessive amounts of new bone are laid down by the increased number of osteoblasts. This osteoblastic proliferation subsequent to the administration of parathyroid hormone is apparently independent of the elevated serum calcium, since it did not occur following injections of calcium only. Increased production of bone under the influence of parathyroid hormone has been reported in growing rats by Bauer, Aub and Albright,¹⁴ Shelling, Asher and Jackson¹⁵ and by Selye,¹⁶ while Burrows¹¹ found increased formation of bone subsequent to a stage of increased resorption.

In growing mice the increased deposition of bone was followed by increased resorption. This reversal led gradually to a normal structure of the diaphysis; but there was no fibrosis of the bone marrow nor any other changes suggestive of fibrous osteitis, as reported in rats, guinea-pigs, rabbits and dogs after administration of parathyroid hormone. Recent biochemical investigations on the phosphatase content in the diaphysis of rats have demonstrated a certain parallelism between histologic and biochemical changes. Roche and Filippi¹⁷ and Williams and Watson¹⁸ injected rats with parathyroid hormone in doses comparable to those we used in mice. They found the phosphatase in the diaphysis increased during the early stages of the experiments, decreased during later stages, and finally back to normal values.

From our experiments no conclusions can be drawn in respect to whether the dose of the hormone might influence the histologic reaction. The data recorded in the literature are not in agreement. Shelling, Asher and Jackson¹⁵ obtained increased formation of bone when low doses of parathyroid hormone had been administered to rats, but increased resorption of bone when high doses had been injected. Jaffe, Bodansky and Blair,¹⁹ on the other hand, observed increased resorption of bone, irrespective of the dose. That very high toxic doses caused cell

TABLE II
*Scheme Demonstrating the Main Effects of Parathyroid Hormone, of Calcium Gluconate
 and of Both Substances on Cartilage and Bone*

		Cartilage		Bone		Onset of epiphyseodiaphyseal union
		Proliferation	Calcification	Formation	Resorption	
A. Conditions in growing mice	Parathyroid hormone	Decreased	Greatly increased	Much increased; strong osteoblastic proliferation	At later stages increased	Accelerated
	Calcium gluconate	Temporarily inhibited	Somewhat increased	Somewhat increased; no osteoblastic proliferation	First decreased; at later stages slightly increased	Unaffected
	Parathyroid hormone and calcium gluconate	Somewhat decreased	Increased	Greatly increased; some osteoblastic proliferation	At later stages somewhat increased	Somewhat accelerated
	Parathyroid hormone		Somewhat increased	Increased	Unaffected	
	Calcium gluconate		Unaffected	Slightly increased	Somewhat decreased	
B. Conditions in adult mice	Parathyroid hormone and calcium gluconate		Strong	Greatly increased	Slightly decreased	

death (necrosis of osteoblasts),²⁰ rather than growth of tissue, is not surprising. However, it is doubtful whether these changes are specific effects of the hormone.

The response to parathyroid hormone depends also on the age of the animal at the beginning of treatment.²¹ In adult mice, the inactive epiphyseal cartilage responded far less to the administration of parathyroid hormone than the growing cartilage of young animals. Moreover, bone formation was intensified only at later stages of the experiment. These findings represent further evidence for the observations reported previously²¹ that longer administration and higher doses of a hormone are required in order to elicit a reaction in older animals. The age factor apparently plays a rôle also in determining the reaction in other species. Old guinea-pigs were found to be far less responsive to the parathyroid hormone than growing animals.²³ Pugsley and Selye²⁴ observed in adult rats, production of bone preceded by intensified resorption of bone, whereas in suckling rats the first reaction to the parathyroid hormone consisted of increased formation of bone.

In growing mice, calcium gluconate likewise accelerated the ageing of the epiphyseal cartilage and favored formation of bone. These findings are in agreement with gross and biochemical observations of increased production of bone in rats,^{25, 26} guinea-pigs,²⁷ and rabbits¹⁴ subsequent to the administration of calcium.

Thus, while the effects of parathyroid hormone and calcium gluconate on cartilage and bone are similar in some respects, they differ in others. As compared with parathyroid hormone, the effects exerted by calcium gluconate were weaker and were demonstrable for shorter periods of time. Moreover, parathyroid hormone caused increased bone formation by means of osteoblastic proliferation. Calcium gluconate, on the other hand, increased the firmness and density of the osseous substance, and thus inhibited its absorption. Finally, the increase of resorptive processes that followed the inhibition of resorption was less prominent in the case of calcium gluconate, whereas it was pronounced in the case of parathyroid hormone.

Notwithstanding the fact that calcium gluconate and parathyroid hormone both accelerated the ageing of the growing epiphyseal cartilage, we did not find an intensification of this effect, if both substances were given simultaneously. This condition may be due to the fact that (1) each substance tends to exert its own peculiar effect on the substratum on which it acts. Consequently, in growing mice, the increased resorption called forth by the parathyroid hormone at later stages is partly counteracted by the inhibiting effect that calcium gluconate exerts on the resorptive processes. Thus, epiphyseo-diaphyseal

union was not only not accelerated, but it was even delayed as compared with the condition found after injections of parathyroid hormone alone. (2) The response of the tissues to hormonal stimuli is partly determined by conditions in the tissues themselves. There is a certain limitation to the tissue response. If the capacity of the cartilage to undergo calcification is exhausted by the administration of parathyroid hormone, an additional supply of calcium does not call forth a further increase in the calcification.

However, the two substances support each other as far as their action on bone is concerned. In this case, a summation effect occurs for a certain length of time; the surplus of calcium available to the proliferating osteoblasts favors the formation of excess bone. Moreover, the inhibition of resorptive processes causes the prolonged persistence of the bone. Thus considerable amounts of excess bone are found at certain experimental stages.

SUMMARY

In mice receiving an adequate amount of dietary calcium, parathyroid hormone promotes the hypertrophy, calcification and disintegration of the growing epiphyseal cartilage without stimulating its proliferation; it intensifies the formation of bone by stimulating osteoblastic proliferation. Subsequently, increased resorption of cartilage and bone causes an accelerated onset of epiphyseo-diaphyseal union. Parathyroid hormone thus promotes the changes that are characteristic of skeletal ageing. In old mice increased calcification of the inactive epiphyseal cartilage is associated with or followed by increased formation of bone.

Calcium gluconate also promotes the ageing of the growing epiphyseal cartilage by increasing disintegration, calcification and ossification. Resorptive processes, however, are temporarily inhibited in both young and adult mice. The effect of calcium gluconate is less marked and of shorter duration than that of parathyroid hormone.

Combined administration of parathyroid hormone and calcium gluconate does not intensify the ageing effect exerted on the growing cartilage by each substance alone. Bone formation, however, is more increased than after administration of each substance alone in both growing and adult mice.

The photomicrographs were made by Mr. Edgar Nebel.

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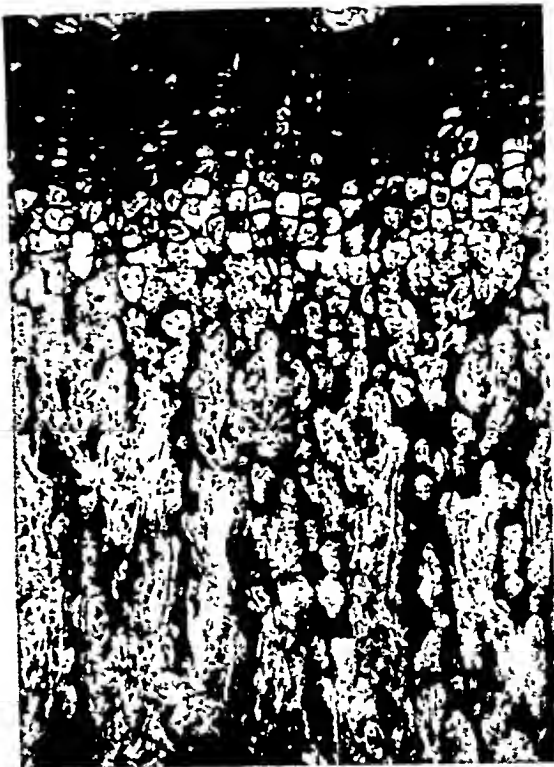
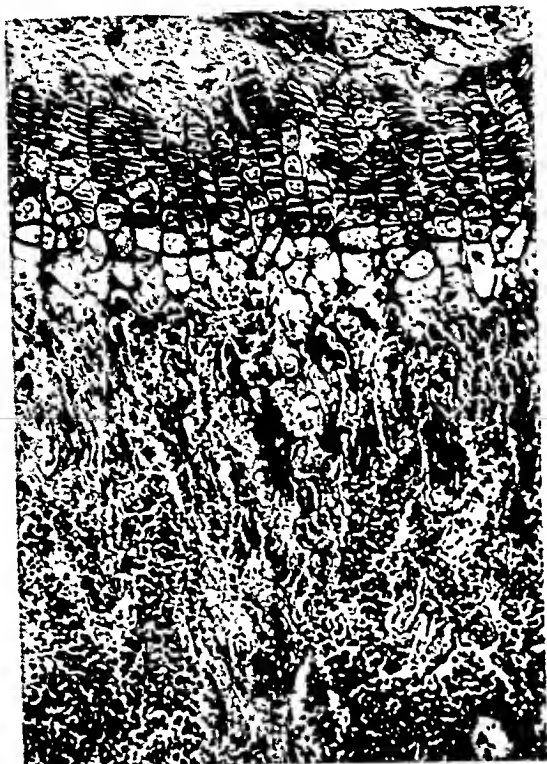
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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 102

- FIG. 1. Epiphyseal disk at the upper tibia of a normal male mouse of strain C57, 7 weeks old. $\times 130$.
- FIG. 2. Epiphyseal disk at the upper tibia of a male mouse of strain C57, which, at the age of 6 weeks, had received 4 units of parathyroid hormone and was sacrificed on the next day. There is increased hypertrophy of the epiphyseal cartilage cells. The subchondral layer contains more spicules; the bone marrow between the trabeculae is replaced by an osteogenic tissue. (See Fig. 5.) $\times 130$.
- FIG. 3. Epiphyseal disk at the upper tibia of a male mouse of strain C57, which, since the age of $6\frac{1}{2}$ weeks, had received 4 units of parathyroid hormone on 4 successive days, and which was sacrificed 1 day after the last injection. Epiphyseal plate is heavily calcified. As compared with Figure 1, trabeculae are more numerous, thickened and densely calcified. $\times 130$.
- FIG. 4. Epiphyseal disk at the upper tibia of a male mouse of strain C57, 10 weeks old, which, since the age of 6 weeks, had received 4 units of parathyroid hormone three times weekly. The greatly narrowed and strongly calcified epiphyseal plate shows perforations leading to communications between the epiphysis and diaphysis. $\times 130$.



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PLATE 103

FIG. 5. Area of the subepiphyseal layer of the animal shown in Figure 2. Mitotic proliferation of the peritrabecular cells is indicated by arrows. $\times 650$.

FIG. 6. Area of the shaft of the animal shown in Figure 2. There is increased formation of spindle cells and of osteoblasts at the inner surface of the shaft. $\times 650$.

FIG. 7. Area of the shaft of the animal shown in Figure 3. There is increased formation of osteoblasts at the inner surface. Endosteal connective tissue cells are proliferating. Mitosis is present. No invasion is seen of the proliferating spindle cells into the layer of osteoblasts, nor into the shaft nor the bone marrow. $\times 650$.

FIG. 8. Area of the shaft of the animal shown in Figure 4. The inner surface is shown lined by a thin layer of spindle cells, as seen ordinarily in untreated animals. $\times 650$.

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PLATE 104

- FIG. 9. Upper tibia of a normal male mouse of strain CBA, 14 months of age. Remnants of hyalinized inactive epiphyseal cartilage are seen, delimited towards the bone marrow by a thin bony lamella. $\times 130$.
- FIG. 10. Upper tibia of a male mouse of strain CBA, 13 months old, which, since the age of 12 months, had received 7 units of parathyroid hormone three times weekly. There is a thickened osseous lamella underneath the heavily calcified cartilaginous remnants of the former growth zone. $\times 130$.
- FIG. 11. Epiphyseal disk at the upper tibia of a male mouse of strain C57, 12 weeks old, which, since the age of 8 weeks, had received injections of 0.25 cc. of a 10 per cent solution of calcium gluconate three times weekly. Hyalinized epiphyseal cartilage showing three plugs of degeneration. $\times 130$.
- FIG. 12. Upper tibia of a male mouse of strain C57, 14 months old, which, since the age of 12 months, had received alternating injections of 7 units of parathyroid hormone and 0.25 cc. of a 10 per cent solution of calcium gluconate three times weekly. There is heavily calcified cartilage in the former growth zone. Much new bone is laid down in the subepiphyseal layer. $\times 130$.

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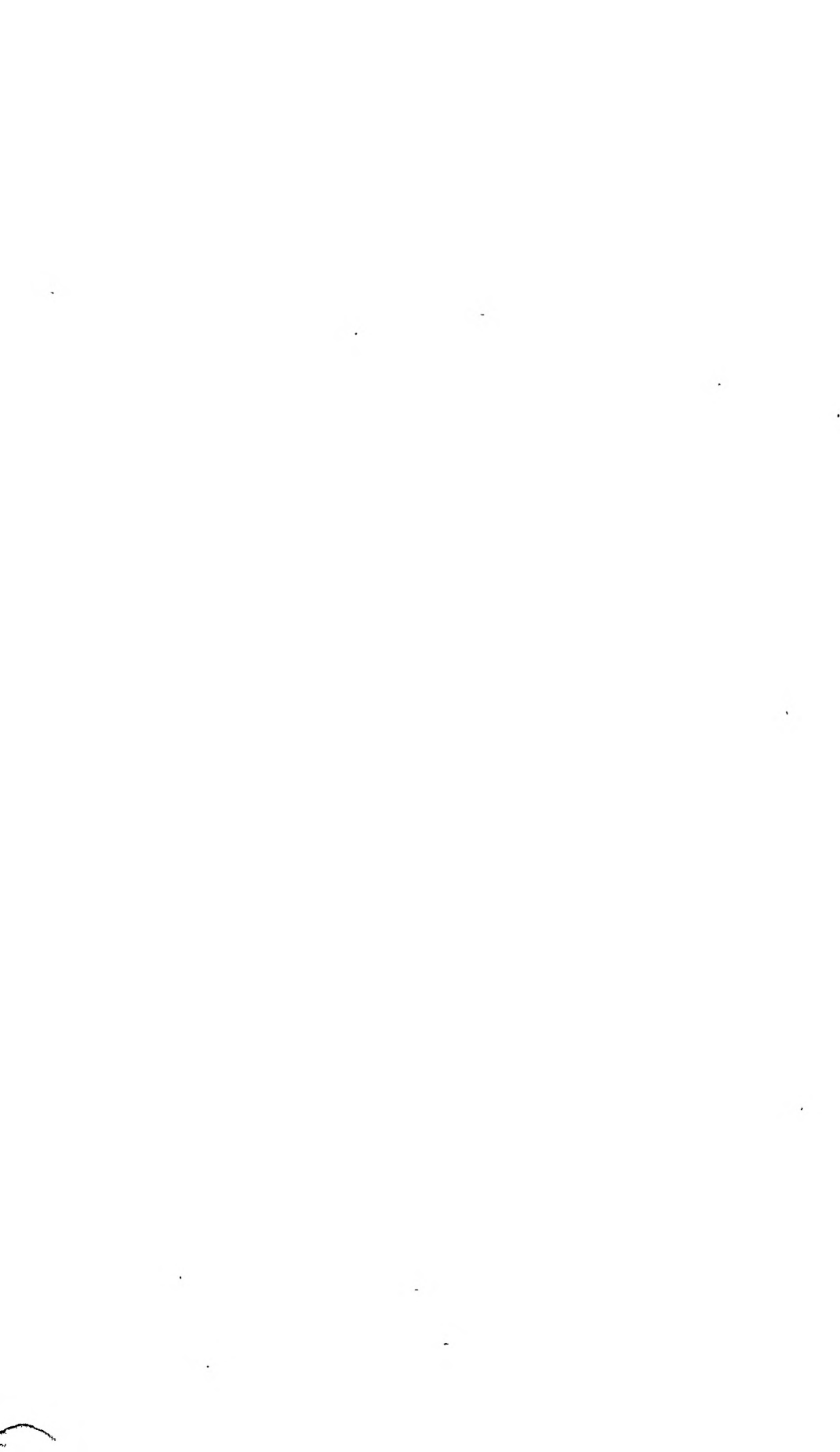


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Parathyroid Hormone and Calcium Gluconate



THE TISSUE CHANGES PRODUCED BY ESTRONE INJECTED INTO FEMALE DOGS WITH BILE FISTULAS *

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The changes produced by large doses of natural and synthetic estrogens in the hematopoietic system, the endocrine system, and the other organs of normal dogs have been described by several investigators.¹⁻⁴ The excretion of injected estrogens in the bile of dogs with bile fistulas⁵⁻⁷ and in the bile obtained from dogs at autopsy⁸ has also been investigated. To our knowledge there have been no reports concerning the anatomic findings following the injection of estrogens into dogs with bile fistulas. In a continuation of a recent study⁶ in which the biliary excretion of estrogens was determined following the injection of a small amount of estrone, six bitches with bile fistulas were employed to investigate the biliary excretion after large injections of estrone.[†] The anatomic findings in these animals are reported in this paper.

MATERIALS AND METHODS

The results obtained in three animals which received subcutaneous injections of estrone in sesame oil are compared with those in three control dogs which received sesame oil only. The details of the preparation of the bile fistulas have been reported.⁶ Each animal received 50 cc. of bile daily by stomach tube. The controls furnished bile for administration to the experimental animals. The letters, BF, in the protocols designate the experimental animals and the letters, BFC, designate the controls.

Unless otherwise stated in the protocols, macroscopic and microscopic examination was carried out on the following tissues: thymus, thyroid, parathyroids, heart, aorta, lungs, spleen, esophagus, stomach, intestines, liver, gallbladder, pancreas, adrenals, kidneys, bladder, uterine horns, ovaries, vagina, lymph nodes, bone marrow (rib, vertebral, femoral), pituitary, subcutaneous tissue at the sites of injection, and the anterior abdominal wall. Those tissues which were considered to be normal are not described specifically in the summary of the anatomic findings. After fixation in Zenker's solution, the tissues were imbedded in paraffin, cut at 6 μ and stained with hematoxylin and eosin. In the case of the spleen, the liver, the lymph nodes and the bone marrow, additional sections were stained with Turnbull's

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† The estrone was obtained through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

blue reaction for the demonstration of iron, and with Giemsa's stain for the study of the cells of the hematopoietic system. Portions of liver and kidneys were also fixed in a 4 per cent solution of formaldehyde, cut at 15μ and stained with scarlet red for the demonstration of neutral fat. The technic of the differential cell count on the rib marrow has been described.⁹ The differential count of the blood leukocytes was done on 200 cells of smears stained with the May-Grünwald-Giemsa stain.

PROTOCOLS OF EXPERIMENTS

Dog BF-3. Weight, 23 lbs. Beginning on the eighth day after operation this dog received daily, for 15 days, 5 mg. of estrone in 1 cc. of sesame oil, a total of 75 mg. Death occurred 2 days after the last injection. Just before death she developed marked lassitude, and vaginal bleeding occurred. There were numerous petechiae in and around the mouth. Her final weight was 18 lbs.

Macroscopic examination revealed the following: a pale, dilated, flabby heart with petechiae on the surface of the right auricle; multiple petechiae and ecchymoses throughout the lungs; a brown-gray-green mottled and friable liver; a thickened, opaque gallbladder; a large right uterine horn and a left uterine horn distended with blood; large, congested mediastinal and mesenteric lymph nodes; pale, tan-gray rib marrow; and anterior abdominal wall tissues which were dark red, swollen and friable.

Microscopic examination disclosed the following: infiltration of the stroma of the right auricle and of the sinusoids of the spleen, liver (Fig. 1), adrenals and lymph nodes by large numbers of cells of the neutrophile granulocyte line varying from promyelocyte to segmented forms; small foci of bronchopneumonia; large amounts of granular, brown, iron-containing pigment in the macrophages and reticulo-endothelial cells of the spleen, liver, lymph nodes and bone marrow; amorphous, brown, iron-free pigment in most of the canaliculi of the liver (Fig. 7); dilatation of the basal glands of the endometrium with increased height of the lining epithelium in which were inclosed nuclear debris and polymorphonuclear neutrophils, focal edema and infiltration of the stroma around the basal glands by polymorphonuclear neutrophils, dilatation of the tubules which were lined with flattened epithelium and in which cellular debris was contained, and infiltration of the crypt layer by lymphocytes, plasma cells, and monocytes; tubes lined by high-columnar ciliated epithelium; ovaries which were about 2 months metestrus—many of the lutein cells of the corpora lutea contained nuclei with between one and five acidophilic inclusion bodies

(Fig. 8), which were distinct from the smaller basophilic nucleoli; a greatly increased myeloid/erythroid ratio, a marrow cell/fat cell ratio of 100/1, and an absence of megakaryocytes in the rib marrow (Fig. 5); and an acute necrotizing, hemorrhagic cellulitis and myositis of the anterior abdominal wall. The determination of the cellular elements of the peripheral blood and a differential count of the cells of the bone marrow were not done for this animal.

Dog BF-4. Weight, 25 lbs. Beginning on the sixth day after operation this dog received daily, for 15 days, 5 mg. of estrone in 1.5 cc. of sesame oil, a total of 75 mg. She was sacrificed on the third day following the last injection. Her final weight was 24 lbs.

Macroscopic examination revealed the following: a pale, flabby heart with petechiae in the epicardium of the right auricle; ecchymoses over the upper lobe of the right lung; brown yellow-green, moderately firm liver; thickened, gray, red mottled gallbladder; gray-red rib and vertebral marrow and yellow, red-stained femoral marrow; and swollen and dark red tissue of the anterior abdominal wall.

Microscopic examination disclosed the following: petechial hemorrhages and small foci of segmented neutrophils in the stroma of the right auricle; petechial hemorrhages and small foci of organizing pneumonia in the lungs; moderate hyperplasia of the lymphatic tissue, and sinusoidal macrophages inclosing granular, brown, iron-containing pigment in the spleen and the lymph nodes; granular, brown, iron-containing pigment in the Kupffer cells of the liver, amorphous, brown, iron-free pigment in many of the bile canaliculi, and fatty metamorphosis of the periportal liver cells; chronic cholecystitis; tall-columnar epithelium lining the basal glands, tubules, crypts and surface, and an edematous crypt layer in the endometrium; tall-columnar ciliated epithelium lining the tubes; ovaries about 2 months anestrus; a lining of stratified squamous epithelium six to seven layers thick and edema of the lamina propria in the vagina; acute, diffuse cellulitis and fibrosis of the anterior abdominal wall; and large empty spaces surrounded by flattened, foamy macrophages, segmented neutrophils, fibrinoid necrotic material and fibrous connective tissue at the site of injection.

Dog BF-6. Weight, 25.7 lbs. Beginning on the 25th day after operation this dog received 5 mg. of estrone in 1.5 cc. of sesame oil daily for 7 days and 6.3 mg. on the 8th day, 41.3 mg. in all. She was sacrificed on the 3rd day after the last injection. The final weight was not recorded.

Macroscopic examination showed the following: a brown, firm liver; a thickened, opaque gallbladder; gray-brown rib marrow, dark red

vertebral marrow and yellow-brown femoral marrow; a gray, indurated anterior abdominal wall; and thickened, boggy, yellow-gray injection sites.

Microscopic examination revealed the following: granular, brown, iron-containing pigment in the macrophages and reticulo-endothelial cells of the spleen, liver, lymph nodes and bone marrow; amorphous, brown, iron-free pigment in the bile canaliculi of the liver; chronic cholecystitis; basal glands lined by tall-columnar epithelium, edema of the stroma, and numerous pigmented macrophages in the endometrium; ovaries in late metestrus; a lining of stratified squamous epithelium and edema of the lamina propria in the vagina; proliferated patches of stratified squamous epithelium from the basal layer (with crowding away of the overlying columnar cells), edema and pigmented macrophages in the lamina propria of the endocervix; and reactions at the injection sites in which the early response showed segmented neutrophils, fibrin, granulation tissue, fresh hemorrhage, connective tissue proliferation and pigmented macrophages, and in which the late response consisted of foreign body giant cells, fibrin, pigmented macrophages, and fibrous connective tissue.

Dog BFC-1. Weight, 15 lbs. Beginning 1 day after operation this dog received 1.0 cc. of sesame oil daily for 21 days. During this time part of her bile was used for feeding a dog with a bile fistula which received estrone, but which was not necropsied. Beginning on the 25th day after operation she was given 1.0 cc. of sesame oil daily for 29 days. She was sacrificed 3 days after the last injection when her weight was 9 lbs.

Macroscopic examination revealed the following: a thickened, gray gallbladder; gray-red rib and vertebral marrow and yellow, red-streaked femoral marrow; and moderately thickened, gray anterior abdominal wall.

Microscopic examination disclosed the following: a few pigmented macrophages in the thymus; slight fatty metamorphosis of the liver cells in the central part of the lobules (Fig. 2); chronic cholecystitis; the uterine horns, ovaries, and vagina in early anestrus; and normoblastic hyperplasia in the rib marrow (Fig. 6).

Dog BFC-3. Beginning 2 days after operation this dog received 1.5 cc. of sesame oil daily for 10 days. She was sacrificed on the tenth day.

Macroscopic examination revealed the following: a pale, tan-red, firm liver; a thickened, opaque gallbladder; dark red rib and vertebral marrow and tan femoral marrow; and a gray, thickened anterior abdominal wall.

Microscopic examination showed the following: varying amounts of granular, brown, iron-containing pigment in the macrophages and reticulo-endothelial cells of the spleen, liver, lymph nodes and bone marrow; amorphous, brown, iron-free pigment plugging some of the bile canaliculi; moderate vacuolation of the liver cells in the central part of the lobules; focal acute cholecystitis; and immature uterine horns, ovaries and vagina.

Dog BFC-4. Beginning 2 days after the operation this dog received 1.5 cc. of sesame oil daily for 9 days. She was sacrificed on the ninth day.

Macroscopic examination disclosed the following: a pale, tan, firm liver; a thickened, opaque gallbladder adherent to the duodenum; dark red rib and vertebral marrow and light tan-red femoral marrow; and a thickened, gray anterior abdominal wall.

Microscopic examination disclosed the following: small foci of bronchopneumonia; chronic cholecystitis; immature uterine horns, ovaries and vagina; acute lymphadenitis of the mediastinal lymph nodes, and scattered pigmented macrophages in the sinusoids of two mesenteric lymph nodes; and many clear spaces surrounded by one or more rows of monocytes at the site of injection.

COMMENT

In Table I is given a comparison of the results of the staining reactions for neutral fat in the liver and kidney, and for pigment in the liver, spleen, lymph nodes and bone marrow. The tissues of the animals which received estrone in oil revealed certain changes which are possibly due to the injections of estrone. The neutral fat content of the liver cells does not appear to differ greatly in the experimental or con-

TABLE I
*Neutral Fat in Liver and Kidney and Pigment in Spleen, Liver,
Lymph Nodes and Bone Marrow*

	Animals injected with estrone in sesame oil			Animals injected with sesame oil alone		
	BF-3	BF-4	BF-6	BFC-1	BFC-3	BFC-4
Neutral fat in liver cells	o	II	o	II	III	o
Neutral fat in cells of cortical portion of renal collecting tubules (Figs. 3 and 4)	V	V	III	I	II	o
Iron-containing pigment in Kupffer cells of liver	V	IV	II	I	II	o
Iron-containing pigment in bone marrow	V	IV	V	I	II	I
Iron-containing pigment in spleen	V	III	III	I	III	I
Iron-free pigment in canaliculi of liver	V	V	II	o	II	o
Iron-containing pigment in lymph nodes	V	IV	V	II	IV	III

Grade I indicates presence; grade V, abundance; and grades II, III, and IV, intermediate amounts of neutral fat and pigment.

trol animals. However, there is an increased amount of neutral fat in the cells of the cortical portions of the renal collecting tubules in those animals which received estrone in oil as compared with those which received oil only. The deposit of iron-containing pigment is definitely greater in the Kupffer cells of the liver, in the bone marrow and in the spleen of those animals which received estrone. The greater quantity of iron-free pigment in the bile canaliculi of the livers of the animals injected with estrone is readily apparent. The amount of iron-containing pigment in the lymph nodes does not differ significantly in the two groups.

Table II contains the data for the last blood picture of all animals except dog BF-3. A striking leukocytosis and shift to the left in the neutrophile granulocyte line is seen only in dog BF-4, probably because this animal received almost twice as much estrone as dog BF-6.

Table III shows the results of the differential count of the cells of the

TABLE II
Data of the Last Examination of the Blood

	Experimental dogs		Control dogs		
	BF-4	BF-6	BFC-1	BFC-3	BFC-4
Hemoglobin (Newcomer), gm.	15.6	12.0	9.2	14.2	11.6
Erythrocytes—millions per cmm.	6.53	4.80	4.64	6.0	5.55
Leukocytes per cmm.	44100	28050	10950	11550	9650
Metamyelocyte neutrophils, %	8.5	0.5	0.0	1.0	3.0
Stab neutrophils, %	40.0	14.5	4.0	14.5	19.0
Segmented neutrophils, %	36.5	70.5	73.0	80.0	56.0
Lymphocytes, %	12.0	12.0	15.0	2.5	20.5
Eosinophils, %	2.0	2.5	7.0	0.5	1.0
Monocytes, %	1.0	0.0	1.0	1.5	0.5

TABLE III
Differential Counts of the Rib Marrow; Cellularity of the Rib, Femoral, and Vertebral Marrows; and Number of Megakaryocytes in the Bone Marrow

	Experimental dogs		Control dogs		
	BF-4	BF-6	BFC-1	BFC-3	BFC-4
Eosinophils, %	1.6	3.2	4.8	2.0	5.4
Promyelocyte neutrophils, %	8.6	4.0	0.4	3.0	1.0
Myelocyte neutrophils, %	24.0	22.2	3.6	12.8	10.2
Metamyelocyte neutrophils, %	34.6	28.2	7.4	16.4	20.2
Stab neutrophils, %	19.6	24.2	14.4	26.8	23.4
Segmented neutrophils, %	0.0	0.8	2.6	5.8	2.8
Pro-erythroblasts, %	0.0	0.2	1.6	0.6	0.2
Erythroblasts, %	1.0	1.6	3.6	3.4	2.0
Normoblasts, %	6.2	11.2	57.6	21.6	29.2
Stem cells, %	0.6	0.6	0.6	0.4	0.0
Lymphocytes, %	1.8	1.2	2.0	3.8	3.4
Unidentified cells, %	2.0	2.6	1.4	3.4	2.2
Myeloid/erythroid ratio	12.3	6.4	0.5	2.6	2.0
Marrow cell/fat cell ratio					
rib	100/1	75/25	90/10	65/35	75/25
vertebra	100/1	75/25	90/10	65/35	65/35
femur	30/70	35/65	10/90	25/75
Megakaryocytes	Absent	Absent	Many	Many	Many

rib marrow, the cellularity of the rib, vertebral and femoral marrows, and the number of megakaryocytes in the bone marrow for all animals except dog BF-3. The rib marrows of dogs BF-4 and BF-6 reveal a marked shift to the left in the neutrophile granulocyte line, a great increase in the myeloid/erythroid ratio, hyperplasia, and an absence of megakaryocytes. These observations confirm those of other workers.¹⁻³

The hyperplasia and normoblastic activity in the marrow of dog BFC-1 may be related to the length of time she carried the bile fistula even though she was fed presumably adequate amounts of bile by stomach tube. The presence of anemia in dogs with bile fistulas has been recently demonstrated.¹⁰

The inflammatory changes in the gallbladder, the fibrosis of the anterior abdominal wall and the fibrosis around the bile drainage tube with the formation of a sinus tract were necessary results of the operative procedure.

In the animals injected with estrone, the high-columnar ciliated epithelium lining the tubes, the evidences of glandular hyperplasia of the endometrium, the edema of the endometrium and of the lamina propria of the vagina, the proliferation of a stratified squamous epithelium in the vagina, the pigmented macrophages in the endometrium, and possibly the intranuclear inclusion bodies in the lutein cells of dog BF-3 may be considered to be results of the action of estrone. Changes of this type were not found to be a part of the normal histologic variations in the ovaries, uterine horns, or vagina.¹¹

Under the conditions of the experiments, the injections had no effect on the pituitary, thyroid, parathyroids, adrenals, or pancreatic islets as judged by the absence of definite histologic alterations in these glands.

The mild inflammatory changes seen in the lungs appear to have no significance when the findings in the two groups are compared.

SUMMARY

The anatomic findings following the injection of estrone in sesame oil into three bitches with bile fistulas were compared with those in three similar animals injected with sesame oil alone. In contrast to the dogs injected with sesame oil alone, those injected with estrone in oil showed the following tissue changes:

1. An increased amount of fat in the cells of the cortical portions of the renal collecting tubules;
2. A greater amount of iron-containing pigment in the Kupffer cells of the liver, in the bone marrow and in the spleen;
3. A larger quantity of iron-free pigment in the bile canaliculi of the liver;

4. Stimulation of granulocytopoiesis and suppression of megakaryocytosis confirming the observations of other investigators;
5. Evidence of estrogenic stimulation of the endometrium, endocervix and vagina;
6. Intranuclear inclusion bodies in the lutein cells of the ovaries of one animal.

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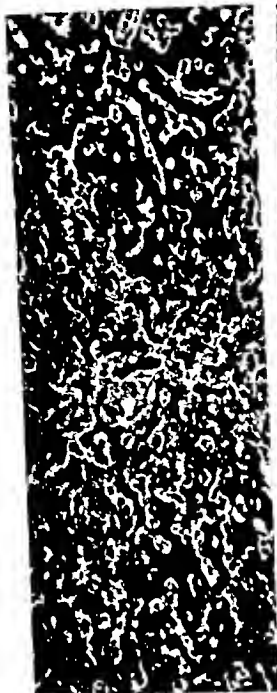
[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 105

- FIG. 1. Liver of dog BF-3. Many young forms of the neutrophile granulocyte line are present in the sinusoids. Hematoxylin and eosin stain. $\times 120$.
- FIG. 2. Liver of dog BFC-1. Sinusoids are practically devoid of leukocytes as compared with Figure 1. There is a fine vacuolation of cytoplasm of many liver cells, a characteristic of abundant glycogen. Coarse fat vacuoles are seen in several liver cells. Hematoxylin and eosin stain. $\times 120$.
- FIG. 3. Kidney of dog BF-3. Black granules represent neutral fat in the cells of the cortical portions of the collecting tubules. Scarlet red stain. $\times 120$.
- FIG. 4. Kidney of dog BFC-1. Black granules represent neutral fat in the cells of the cortical portions of the collecting tubules. Scarlet red stain. $\times 120$.
- FIG. 5. Rib marrow of dog BF-3. Representative area showing marked hyperplasia, absence of megakaryocytes and preponderance of young forms of the neutrophile granulocyte line. Hematoxylin and eosin stain. $\times 600$.
- FIG. 6. Rib marrow of dog BFC-1. Representative area showing hyperplasia, many normoblasts, a well preserved megakaryocyte and more mature forms of the neutrophile granulocyte line. Hematoxylin and eosin stain. $\times 600$.
- FIG. 7. Liver of dog BF-3. Many young forms of the neutrophile granulocyte line in the sinusoids. Arrows in the upper half of the field point to bile canaliculi plugged with pigment. The arrow in the lower half points to a Kupffer cell loaded with pigment. Hematoxylin and eosin stain. $\times 600$.
- FIG. 8. Corpus luteum, ovary of dog BF-3. The arrow in the upper half of the field points to a lutein cell nucleus with two inclusion bodies. The arrow in the lower half points to a lutein cell nucleus, with inclusion body in the lower portion and nucleolus in the upper portion. Hematoxylin and eosin stain. $\times 600$.

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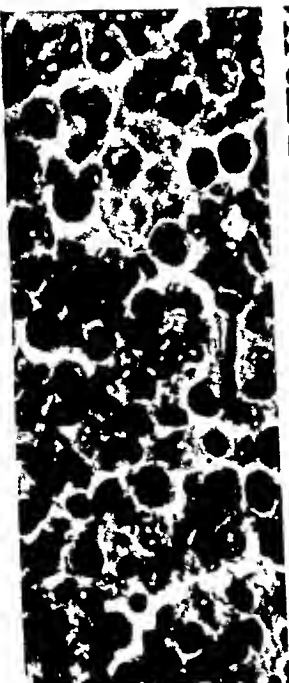
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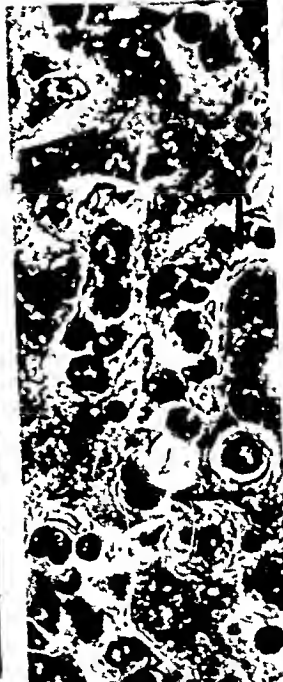
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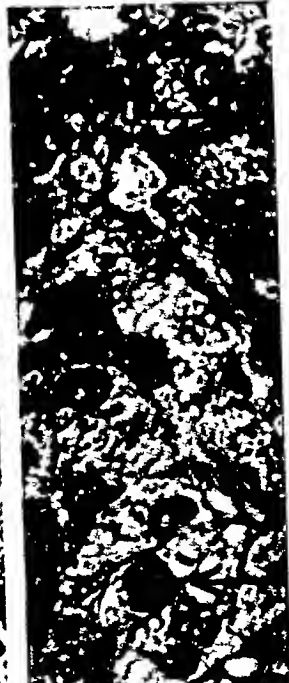
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Mulligan, Longwell and Morrell

Estrone in Dogs with Bile Fistulas

THE NATURE OF THE HYALINE MATERIAL IN THE PANCREATIC ISLANDS IN DIABETES MELLITUS *

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The histopathology of diabetes mellitus, although extensively investigated, must still be considered an unconquered territory since, of the many changes described, none has as yet been proved conclusively to be the responsible manifestation of diabetes. Most of the reports on this subject have failed to differentiate between adult and juvenile diabetes which, of course, has added to the confusion. Keilty¹ and Weichselbaum and Stangl² considered grossly recognizable pancreatic atrophy a significant factor in diabetes mellitus, while Cecil³ found a reduction in the size of the pancreas in only 25 per cent of his cases. Warren,⁴ in a study of 484 diabetic patients, reported the following pancreatic changes: hyalinization, 200 cases; fibrosis, 129; normal pancreas, 127; hydropic changes, 22; lymphocytic infiltration, 9; calculi, 3; amyloid, 2. Martius⁵ reported the following island changes in diabetes mellitus in order of frequency: sclerosis, hyalinization, hydropic degeneration, simple atrophy, hemorrhage. Interstitial pancreatitis was found by Gibb and Logan⁶ in 123 of 147 cases of diabetes mellitus. Herzog⁷ interpreted multiple fibrous nodules in the pancreas of a diabetic person as remnants of islands. Wilder⁸ called attention to the more frequent occurrence of lipoids in the islands of diabetic persons than of those without diabetes. Boldyreff⁹ reported a case of occlusion of the pancreatic duct resulting in death in diabetic coma years later; the pancreas in this case showed extreme atrophy with disappearance of the island tissue. In contrast to this report. Umber,¹⁰ in a very similar case, found marked regeneration of the islands without existence of diabetes mellitus. A segregation of cases of juvenile diabetes from those of diabetes of the higher age groups was made by Glen,¹¹ who found island changes to be rare in the former and frequent in the latter group. Labbé and Pétresco¹² found sclerosis of the islands more frequent in the middle-aged group while hydropic and pyknotic islands were more predominant in the younger patients.

PURPOSE OF PRESENT INVESTIGATION

The purpose of this investigation is to demonstrate that the term "hyalinization" in reference to the islands of Langerhans is misleading since the hyaline material frequently found in the pancreatic islands

* Received for publication, December 31, 1942.

shows staining properties identical with those of amyloid. If special stains are employed, it can be demonstrated that these changes are extremely frequent and appear where routine hematoxylin and eosin stains have failed to reveal any pathology.

Only a few reports in the literature mention amyloid in reference to the pancreatic islands. Opie,¹³ in 1900, reported a case of hyaline degeneration of the pancreatic islands in which he mentioned specifically that the reactions for amyloid were negative for this hyaline material. In 1905, Reitmann¹⁴ reported two fatal cases of pulmonary tuberculosis in a middle-aged male and a young female in whom amyloid was found in the islands of Langerhans but nowhere else; the author's description suggests that the process in these cases was histologically indistinguishable from hyalinization of the islands. In 1937, Warren⁴ pointed out that hyalinization of the islands is caused by the deposition of a substance which is "related to or possibly identical with amyloid." In his review of pancreases of 484 diabetic persons he found hyaline islands in 200, with 97 per cent in persons over 40 years of age. Of 51 of these cases, tissue from 14 gave positive staining reactions for amyloid. Although Warren suggested the amyloid theory of insular hyalin, he expressed doubts as to its value because of negative amyloid stains in the majority of his cases.

In contrast to Warren's⁴ observations, none of the cases presented in this investigation failed to show positive amyloid stains in the hyaline material deposited in the pancreatic islands. Van Gieson's stain gave the same shade of yellow which it produced on known amyloid tissue. The same van Gieson's mixture applied to ovarian corpora fibrosa yielded the usual pink stain of hyaline connective tissue. The regularity with which the hyaline material of the pancreatic islands gave a positive stain for amyloid seems to prove that this substance is at least related to, and in all probability, identical with, amyloid. Lacking conclusive microchemical means of identification, it seems justified to consider this hyaline material to be amyloid until it can be disproved as such.

MATERIAL AND METHODS

The present material * was derived from 157 autopsied cases: 105 middle-aged or old diabetic patients; 26 juvenile diabetic patients; and 26 nondiabetic patients with island changes found upon routine microscopic examination. The material consisted of formaldehyde-fixed paraffin blocks, usually two or three but in a few cases only one, from each pancreas. From every block sections were stained in hematoxylin

* Obtained from the Department of Pathology, University of Michigan, Ann Arbor, Michigan.

and eosin and with special stains for amyloid. It was found that the most practical method for the demonstration of amyloid was staining with gentian violet followed by differentiation in diluted acetic acid. After proper differentiation the slides were rinsed in tap water and examined immediately while they were still wet, without the use of coverslips. By this method the hyaline material stood out as a brilliant red substance, facilitating detection of even the smallest number of hyaline islands. Rechecks later on, however, required complete re-

TABLE I
Number of Patients with and without Amyloid in the Islands of Langerhans

	Amyloid-positive	Amyloid-negative	Total
Juvenile diabetic patients	1	25	26
Middle-aged or old diabetic patients	67	38	105
Nondiabetic patients with recorded islet pathology	26		26
Nondiabetic patients over 50 years of age selected from consecutive autopsy cases	5	45	50

staining. The microscopic studies were made with artificial light, as the natural light decreased the brilliancy of the red color, giving the hyaline material a slightly bluish shade and making its differentiation from the surrounding blue tissue more difficult.

It was found that the occurrence of amyloid varied from one amyloid island in a single level of the pancreas to complete amyloidosis of almost all islet structures. Accordingly, it was attempted to classify these changes arbitrarily by the designation of 1, 2, and 3 plus. Obviously, this quantitative classification was of but little accuracy as it was based on a more or less subjective impression and on the examination of only one, two, or, at the most, three levels of the pancreas; however, for practical purposes it seemed to suffice. Quantitative amyloid changes within individual islets were also noted but not specifically evaluated.

RESULTS OF CASE STUDY

Pancreases of 131 diabetic persons were studied, 26 being cases of juvenile diabetes. Of the 105 middle-aged or older diabetic patients, the pancreases of 67, or 64 per cent, showed amyloid in the islands of Langerhans to varying degrees. Of the 26 juvenile diabetic cases, only 1 pancreas showed amyloid. In addition, the pancreases of 26 nondiabetic patients were examined for which, in the routine microscopic examination of the organs, hyaline changes of the islands were recorded; all of these 26 gave positive amyloid stains of the hyaline material. In-

asmuch as these amyloid-positive nondiabetic cases did not give any information as to the total incidence of this process, the pancreases of 50 consecutive autopsy cases of nondiabetic patients over 50 years of age were examined. Five of these cases (10 per cent) revealed the same amyloid changes, although the involvement was usually slight.

It did not seem justified to separate middle-aged and old diabetic patients, as there are no grounds for such separation from a pathologic point of view. The series of juvenile diabetes was omitted from further investigation as these cases were believed to represent a different disease entity, clinically as well as pathologically. It is true that insular amyloid does occur in cases of juvenile diabetes but these cases are, apparently, rare. In one such instance found in this material, as well as in two similar cases reported by Reitmann,¹⁴ the patients died from advanced pulmonary tuberculosis, which might account for the presence of amyloid in the pancreatic islands.

CLINICAL ASPECTS OF INVESTIGATED CASES

An attempt was made to investigate the clinical relationship between cases with and those without amyloid by a study of the case histories. In this attempt a number of difficulties were encountered because in many instances I had to deal with terminal conditions. Unless the patient had been under observation for some time, the report of a low blood pressure shortly prior to death was of no value; in some cases a left-sided cardiac hypertrophy recorded in the autopsy protocol had to be considered as an indication of a pre-existing hypertension. More difficult was the important evaluation of the body weight, as an apparent weight loss was not always properly recorded. Duration of the disease in most cases had to be estimated from the appearance of suggestive symptoms, such as polyuria or polydipsia, as recorded in the patient's history.

Table II is self-explanatory. While in general the findings offer no conclusive clues in regard to the relationship between amyloid in the islands of Langerhans and diabetes mellitus, there are a few points of interest: (1) Obesity is more common in the diabetic patient with than without amyloid. (2) Amyloid changes in nondiabetic patients are not very common in females. (3) Gangrene of the lower extremities is as common in diabetic patients with amyloid changes as in those showing none. (4) Racial disposition is of no significance in this investigation. (5) Thyroid disease is found in some diabetic persons without amyloid. (6) Hypertension is surprisingly common in connection with amyloid pancreatic islands.

It was noted that the average age of the obese amyloid-positive pa-

tients regardless of absence or presence of diabetes mellitus was distinctly lower than that of the nonobese amyloid-negative diabetic patients. This point may throw an interesting light on the problem of "glycosuria of the obese middle-aged individual"; there is apparently a greater tendency for patients in this group to show amyloid in the pancreatic islands than for those in the group of nonobese older persons.

The duration of the diabetic condition has, apparently, no influence on the presence or absence of pancreatic amyloid. Dry and Tessmer's¹⁵

TABLE II
Clinical Aspects of Investigated Cases

	Diabetic patients with amyloid in islands	Diabetic patients without amyloid in islands	Nondiabetic patients with amyloid in islands
Number of cases	67	38	26
Males	27	19	22
Females	40	19	4
Percentage of obesity ("obese" or weight of 180 lbs. or more)	54%	36%	45.4%
Average age of "obese" patients	59.3 yrs.	58.3 yrs.	59.2 yrs.
Average age of "nonobese" patients	64 yrs.	59.2 yrs.	65 yrs.
Percentage of patients with systolic blood pressure of 150 or more	40%	28%	50%
Average duration of diabetes	6.2 yrs.	5.7 yrs.	
Previous insulin treatment	14	14	.
Diabetes in family	5	5	2
Racial factors	1	3	0
Gangrene of feet or legs	14	8	2
Infections without gangrene	15	6	3
Evidence of thyroid disease	1	3	1

report on an increase in hyaline changes in the post-insulin era over the pre-insulin era cannot be confirmed from the standpoint of the present investigation. Familial history of diabetes does not seem to favor nor exclude the development of amyloid.

Amyloid in the Pancreas in Generalized Amyloidosis

In 11 cases of generalized amyloidosis in nondiabetic persons the pancreases were examined for amyloid. Nine of these cases showed no amyloid in the islands while 2 showed slight involvement (1 plus); all but 2, however, showed amyloid in the arterioles. A second series of 11 cases, all 3 plus positive for amyloid in the islands, was selected from the original material and examined for amyloid in liver, kidneys, adrenals and spleen. Except for amyloid changes in the pancreatic arterioles, no amyloid was found outside of the islands in any of these cases. Amyloid deposits in the small arteries of the pancreas were commonly found in all cases in this investigation. They were observed in many amyloid-positive diabetic and nondiabetic patients and in a

considerable number of diabetic persons who were negative for amyloid in the islets.

Quantitative Amyloid Changes

Results of an attempt to correlate clinical data with the various degrees of islet amyloidosis are shown in Table III. The data obtained did not reveal differences that could be considered significant, but it must be emphasized again that this classification into three groups is based merely on arbitrary standards and on the findings in a limited

TABLE III
*Clinical Aspects of Investigated Cases Arranged According to
Degrees of Amyloidosis of the Islands of Langerhans*

	Diabetic patients				Nondiabetic patients		
	No amyloid	One plus	Two plus	Three plus	One plus	Two plus	Three plus
Average age in years	58.3	60	60	57	60	62	66.5
Males	19	5	11	11	3	8	10
Females	19	16	14	11	1	1	2
Weight of 180 lbs. and over	23	10	13	6	1	5	4
Weight of less than 180 lbs.	13	9	9	13	4	8	10
Blood pressure of 150 and more systolic	11	10	13	7	2	3	7
Blood pressure of less than 150 systolic	21	7	10	12	2	4	4
Insulin treatment	14	4	2	8			
No insulin treatment	14	12	16	13			
Average duration of diabetes in years	5.5	6.2	5.9	8.3			
Gangrene	8	3	6	5			2
No gangrene	30	16	19	17			10

number of levels of the pancreas. It does not rule out the likelihood that systematic studies, if they were possible, might shift the data of Table III. In the material available for investigation a quantitative classification seems without value.

COMMENT

The principal findings in this investigation are as follows: (1) The term "hyaline fibrosis" of the islands of Langerhans is a morphologic misinterpretation. The hyaline substance found frequently in the pancreatic islands is not connective tissue hyalin but, because of its staining reactions, is believed to be amyloid. (2) Sixty-four per cent of middle-aged or older diabetic patients and 10 per cent of nondiabetic patients of 50 years or over show amyloid of varying degree in the islands of Langerhans. (3) Amyloid is found isolated in the islands without amyloid involvement of other organs, while in cases of generalized amyloidosis the islands are usually not involved at all. (4) Hyper-

tension is commonly found in amyloid-positive diabetic and nondiabetic patients.

Inasmuch as amyloid is more than six times as common in the pancreases of diabetic persons as of nondiabetic persons, a relationship between insular amyloid and diabetes mellitus seems probable. In determining the nature of this relationship it should not be forgotten that: (1) approximately one-third of all cases of diabetes mellitus do not show insular amyloid; (2) in some amyloid-positive cases the number of involved islands is extremely small; and (3) a certain number of persons show insular amyloid without being diabetic. It seems impossible to reconcile these facts with the theory that insular amyloid causes diabetes mellitus. It seems more reasonable to consider amyloid degeneration the result rather than the cause of diabetes and to assume that the pancreatic islands may or may not undergo such degeneration subsequent to pre-existing nondemonstrable changes. Whether the nondiabetic group with insular amyloid should be regarded as being potentially diabetic, as suggested by Warren,⁴ is difficult to determine as long as we have to deal with post-mortem material. Some resemblances to the amyloid-positive diabetic series, such as the high incidence of hypertension, examples of familial history of diabetes and the occurrence of gangrene of the lower extremities, might be considered as supporting such a statement.

The large number of hypertensive cases in the amyloid-positive, nondiabetic series seems quite surprising. Offhand no apparent relationship can be elicited between these two findings from a physiologic point of view. Looking at the problem from a purely histologic angle, however, it may prove of interest to consider the resemblance between renal glomeruli and islands of Langerhans and the relationship of the former to hypertension. It is only one step further to suggest that a pathologic condition of the highly vascular islands of Langerhans may interfere with the circulation very much as do diseased renal glomeruli. The affinity of amyloid for small blood vessels supports this conception in so far as the amyloid seems to be laid down about the endothelial structures first.

If I am correct in my assumption of a relationship between diabetes mellitus and insular amyloid, the question comes up: what causes these nondemonstrable changes in diabetes mellitus which may or may not be followed by amyloid degeneration of the islands of Langerhans? Considering juvenile diabetes a disease entity of a separate nature, diabetes mellitus is a disease of the middle-aged or older person, which points to a possible arteriosclerotic etiology. Weichselbaum and Stangl² found arteriosclerosis of the pancreatic vessels in diabetic persons over

50 years of age. Allen ¹⁶ stated that he does not believe that a decrease of arterial blood supply can cause diabetes mellitus. Warren ⁴ emphasized that in most cases of diabetes mellitus the pancreatic vessels are of sufficient caliber to insure adequate blood supply.

It seems that the vascular etiology of diabetes mellitus cannot be discussed merely on the basis of absence or presence of sclerosis of pancreatic arteries and arterioles but that the blood supply of the pancreas as a whole should be considered. With the bulk of the islet tissue lying in the tail of the pancreas, it should be kept in mind that the head and the tail of the pancreas draw their blood supplies from two different sources: while the head is largely supplied by the superior and inferior pancreaticoduodenal arteries, the tail receives most of its blood through the pancreatic branches of the splenic artery. Thus, the vascular island tissue draws its blood supply mostly from the splenic artery. It is quite conceivable that arteriosclerosis of the splenic artery might greatly interfere with adequate blood supply to the tail of the pancreas by blocking the rather small openings of the pancreatic branches. Thus, this interference may take place outside of the pancreas without manifestations of arteriosclerosis in the more distal portions of the pancreatic blood supply. A relationship between insular amyloid and the splenic circulation is suggested by Warren's ⁴ observation of amyloid in the splenic arterioles in those of his cases which showed positive amyloid reactions in the hyalinized islands.

SUMMARY

1. The change in the islands of Langerhans known as hyaline fibrosis has been misinterpreted histologically and, in all probability, is a deposition of amyloid.

2. In 67 of 105 middle-aged and older diabetic persons (64 per cent), amyloid deposition of varying degrees could be demonstrated in the islands of Langerhans.

3. Similar changes were found in 5 out of 50 consecutive autopsies upon nondiabetic patients over 50 years of age.

4. Amyloidosis of the islands of Langerhans is an isolated feature and, as a rule, is not found in generalized amyloidosis.

5. Amyloidosis of the islands of Langerhans cannot be considered a pathognomonic evidence of diabetes mellitus nor its cause. It is suggested that nondemonstrable changes in diabetes mellitus and, possibly, in pre-diabetic states render the islands susceptible to amyloid deposition.

6. In a high percentage of cases amyloidosis of the islands of Langerhans in both diabetic and nondiabetic persons is associated with

hypertension. The morphologic resemblance between islands of Langerhans and renal glomeruli suggests interference with the circulation in the highly vascular islands as a possible cause of increased blood pressure, in a manner comparable to that resulting in renal hypertension.

7. The percentage of obese patients is slightly higher and their average age lower in the amyloid-positive than in the nonamyloid group; in other words, there is a greater tendency in "glycosuria of the middle-aged obese individual" towards insular amyloidosis than in the older, nonobese patient.

The author is indebted to Dr. L. H. Newburgh and Dr. C. V. Weller of the University of Michigan for their helpful support.

After this paper was set in type, the article by J. B. Arey (Nature of the hyaline changes in islands of Langerhans in diabetes mellitus. *Arch. Path.*, 1943, 36, 32-38) appeared. Both results and conclusions are in substantial agreement with those reported here.

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EFFECT OF VITAMIN E THERAPY ON THE CENTRAL NERVOUS SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS *

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Until recently it was universally recognized that amyotrophic lateral sclerosis is not amenable to any form of treatment. Wechsler,¹ on the basis of experimental studies of animals that were deprived of vitamin E and which developed paralysis and atrophies (Ringsted,² Lipshutz,³ Burr, Brown and Moseley,⁴ Einarson and Ringsted⁵), believed that patients suffering from amyotrophic lateral sclerosis might respond to a synthetic preparation containing alpha-tocopherol. Wechsler,¹ Rosenberger⁶ and Bicknell⁷ found that alpha-tocopherol and natural vitamin E act favorably in some cases of amyotrophic lateral sclerosis and bring about varying degrees of improvement, perhaps in inverse ratio to the age and duration of the disease. Doyle and Merritt,⁸ Denker and Scheinman⁹ and Ferrebee, Klingman and Frantz,¹⁰ however, using the same form of treatment, were unable to produce improvement or to arrest the course of the illness in patients with amyotrophic lateral sclerosis.

A number of cases of amyotrophic lateral sclerosis were treated at the Montefiore Hospital with vitamin E and alpha-tocopherol. Ten of these cases came to necropsy and, except for one, none had responded clinically to this form of treatment. The age, duration of the illness and the fact that they may not have been treated adequately may be used as arguments against the lack of improvement. In this presentation, emphasis will be placed on the possible influence of this form of treatment on the affected structures of the central nervous system and not on the clinical results. The 10 cases of amyotrophic lateral sclerosis that received vitamin E were investigated histopathologically and compared with material from about 40 untreated cases. As will be demonstrated, in many of the treated cases the destruction of myelin sheaths and axis cylinders was less intense than in the untreated cases, while the dense gliosis which is usually present in amyotrophic lateral sclerosis was diminished or almost absent in those that received vitamin E. The anterior horn cells and the nerve cells of the involved bulbar nuclei remained unchanged and showed no signs of reversibility.

* Read at the Combined Meeting of the New York Neurological Society and the Section of Neurology and Psychiatry on October 6, 1942, at the New York Academy of Medicine.

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TABLE I
Cases Treated with Vitamin E, Including Alpha-Tocopherol Intramuscularly, Showing Significant Changes in the Involved Pathways

Case no.	Sex	Age	Duration of illness	Treatment with alpha-tocopherol	Bulbar and anterior horn cell disease	Pyramidal tract signs	Histopathologic findings
1	M	64	1 yr., 10 mos.	7½ months	Bulbar symptoms; marked atrophy and fibrillations of muscles, especially in the distal parts of the extremities	Hyperactive deep reflexes; diminished abdominal and cremasteric reflexes; no Babinski or allied signs	Myelin sheath: no demyelination noted High power examination: slight changes, insular Bielschowsky preparation: slight changes Fat: scarcely any Holzer preparation: very faint gliosis, insular
2	M	60	2 yrs. 7 mos.	1 month and 4 days	Bulbar symptoms; severe atrophy and fibrillations of the muscles of the distal parts of the extremities	Bilateral foot drop; hyperactive deep reflexes with diminished abdominal and cremasteric reflexes, but no other pathologic reflexes	Myelin sheath: faint demyelination with small islands of myelin sheath swelling and destruction, axis cylinders slightly less than in ordinary cases Fat: less fat than normally Holzer preparation: faint gliosis
3	M	44	6 yrs	4 months and 5 days; treated intensively	Severe atrophy and fibrillations of the muscles of the extremities	Spastic gait with marked hyperreflexia in the upper and depressed in the lower extremities; diminished abdominal and absent cremasteric reflexes	Myelin sheath: scarcely any pal- lor High power examination: slight disintegration of fibers, insular Bielschowsky preparation: slight disintegration Fat: present, not as much as in ordinary cases Holzer preparation: slight gliosis, insular

4	F	53	1½ yrs.	7 months and 3 weeks	Bulbar symptoms; atrophy and fibrillations of the muscles	Spastic gait; areflexia except for slight knee-jerk; absent abdominal reflexes and presence of a left Babinski sign	Myelin sheath: no visible demyelination High power examination: slight disintegration of fibers, insular disintegration Bielschowsky preparation: slight disintegration Fat: very little Holzer preparation: faint gliosis, insular
5	F	70	1 yr., 1 mo.	2 months and 7 days	Bulbar symptoms; atrophy and fibrillations of the muscles of the upper extremities	Hyperactive reflexes with bilateral Hoffmann signs and defective plantar responses	Myelin sheath: very slight demyelination High power examination: slight changes, insular Bielschowsky preparation: slight changes Fat: scarcely any Holzer preparation: faint gliosis, insular
6	M	54	2 yrs.	5 weeks and 3 days; received treatment also on the outside, but could not determine exact amount; this patient showed slight improvement in neurologic symptoms	Bulbar symptoms; atrophy and fibrillations of the muscles of the upper extremities	Generalized hyperreflexia with bilateral Hoffmann signs, patellar and ankle clonus	Myelin sheath: slight pallor of left and slight demyelination of right crossed pyramidal tract High power examination: destruction; however, not as marked as in average case; more marked on the right Fat: same as in ordinary cases Bielschowsky preparation: destruction, but less than in ordinary cases, especially on the right Holzer preparation: moderate gliosis in right crossed pyramidal and very slight on the left

TABLE II
Cases Treated with Vitamin E, Some Inadequately, without Significant Changes in the Involved Pathways

Case no.	Sex	Age	Duration of illness	Treatment with alpha-tocopherol	Bulbar and anterior horn cell disease	Pyramidal tract signs	Histopathologic picture
7	M	53	1 yr., 11 mos.	15 days	Atrophy and fibrillations of muscles	Spastic gait; hyperactive deep reflexes; absent abdominal reflexes and defective plantar responses	Myelin sheath and axis cylinder: glial reaction and deposition of fat not much different from ordinary cases of amyotrophic lateral sclerosis
8	M	50	2½ yrs.	3 mos. 10 days	Bulbar symptoms; atrophy and fibrillations of muscles	Hyperactive deep reflexes with bilateral Hoffmann, Rossolimo and Babinski signs	Myelin sheath: moderate destruction Axis cylinder: moderate destruction Fat: some fat droplets, but not as much as in usual cases Holzer preparation: moderate gliosis
9	F	49	2 yrs.	1 yr.; fairly intensive treatment but with interruptions	Bulbar symptoms; atrophy and fibrillations of the muscles of the upper extremities	Spastic gait; hyperactive reflexes in the upper extremities with bilateral Hoffmann signs but diminished reflexes in the lower extremities with no pathologic reflexes	Myelin sheath: demyelination and disintegration of myelin sheaths Fat: same as average Bielschowsky preparation: about same as average case Holzer preparation: moderate gliosis, slightly less than average
10	F	62	2½ yrs.	7 mos., with some interruptions	Atrophy and fibrillations in the upper extremities	Spastic gait; generalized hyperreflexia with bilateral Hoffmann signs; ankle clonus and absent abdominal reflexes	This case did not differ neuropathologically from untreated cases of amyotrophic lateral sclerosis

METHODS OF PROCEDURE

Ten cases (Tables I and II) of amyotrophic lateral sclerosis that received vitamin E in the form of ephynal, alpha-tocopherol, a diet rich in vitamin E, thiamine chloride, wholewheat germ oil and bile salts form the basis of this presentation. Two patients received ephynal by mouth without intramuscular injections of alpha-tocopherol. The treatment in the other eight cases conformed to that outlined by Wechsler.¹

Sections from various cortical areas (where available), internal capsule, peduncles, pons, medulla oblongata and spinal cord were embedded in parlodion and stained by the myelin sheath and cresyl violet methods. Frozen sections were stained by the myelin sheath, Bielschowsky, Sudan III and Holzer methods. For purposes of comparison the gliosis in the untreated cases was designated as dense, while in the treated cases there were gradations which were divided into moderate, slight, or very slight gliosis.

ANALYSIS OF MATERIAL

Clinical Evaluation

These cases (Tables I and II) present a few clinical facts worth discussing. There were 6 males and 4 females, a distribution which conforms with observations that the incidence of amyotrophic lateral sclerosis is greater in males than females. The ages of these patients were: 2 in the fifth decade, 4 in the sixth, 3 in the seventh, and 1 in the eighth. The duration of the illness in most instances was between 2 and 2½ years, except that one patient (case 3) lived 6 years after the onset of the illness and that three patients (cases 1, 4 and 5) lived 20, 18 and 13 months respectively.

Of the ten patients, two (cases 7 and 8, Table II) received ephynal by mouth; the others also received alpha-tocopherol intramuscularly. Case 7 received the treatment only for a period of 15 days. The others were treated for from 5 weeks to 7½ months. Cases 9 and 10 were treated for 1 year and 7 months respectively, but with interruptions. As will be seen, this may have had some relationship to the histopathologic changes. None of these patients except case 6 showed any improvement in the neurologic symptoms.

The first six treated cases that showed a different histopathologic picture from the usual picture in nontreated cases disclosed severe atrophy and fibrillations of the muscles, especially in the distal parts of the extremities, more so in the upper than in the lower (Table I). The pyramidal tract signs consisted of a generalized hyperreflexia in all, diminished or absent abdominal reflexes in cases 1, 2, 3, 5 and 6 (Table I), Hoffmann's sign in cases 5 and 6, Babinski's or allied signs

only in case 4. These cases ought not to be confused with those of progressive spinal muscular atrophy, a disease of the anterior horn cells without pyramidal tract lesions in which there is widespread atrophy of muscles and absent or markedly diminished deep reflexes. Furthermore, the onset of the illness in progressive spinal muscular atrophy starts at a much earlier age and lasts much longer than in amyotrophic lateral sclerosis, at times from 10 to 20 years. Wilson¹¹ and others recognized that atrophy of muscles in amyotrophic lateral sclerosis may become so extreme as to abolish or decrease the respective reflexes: "There is as it were a conflict between the respective tone-increasing and tone-reducing influences of supranuclear and nuclear lesions." In an analysis of 36 other cases of amyotrophic lateral sclerosis that came to necropsy, 15 cases with severe demyelination of the pyramidal tracts because of the marked atrophies had diminished or absent reflexes. It should also be emphasized that most of the patients in this presentation, as in the untreated cases of amyotrophic lateral sclerosis, died because of involvement of the bulbar nuclei, a process which rarely occurs in progressive spinal muscular atrophy.

Wilson,¹¹ who is inclined to place progressive spinal muscular atrophy and amyotrophic lateral sclerosis in the same group, stated:

"Difficult as it is to reach a firm conclusion, the lesions of the Charcot type [amyotrophic lateral sclerosis] are so characteristic, and, when advanced, so different from those of nuclear amyotrophy [progressive spinal muscular atrophy], that in my opinion the fact outweighs other considerations; they comprise much more than the mere addition of upper to lower motor neuron disease. While I consider this divergence more striking than the similarities, the occurrence of gradations from one to the other, and also in the direction of 'subacute anterior poliomyelitis,' must be admitted; not all of either kind run true to type. For *descriptive purposes* [not italicized in the original], however, it is best to take the two varieties together, inclusive of intermediate and aberrant clinical forms."

Histopathologic Evaluations

Nerve Cell Changes. The histopathologic changes in the involved nerve cells of the medulla oblongata (usually 10th and 12th nuclei) and anterior horn cells in the spinal cord were the same in the treated as in the untreated cases of amyotrophic lateral sclerosis. This was true both in regard to the number and to the character of the nerve cell changes. The involvement of the bulbar nuclei and the progress of the changes in these nerve cells resulted in death in most of these cases.

Pathway Changes. On the other hand, definite differences were present in the myelin sheaths, axis cylinders, fat and glia.

In cases 1, 2, 3, 4, 5 and 6 (Table I), or 60 per cent of the cases, the pathologic process in the involved pathways of the central nervous

system, especially of the spinal cord, differed entirely from the untreated cases. In these, except case 6, demyelination of the crossed pyramidal tracts in the myelin sheath preparation could hardly be detected with the naked eye (Fig. 1). This was not true in the untreated cases where the demyelination stands out prominently (Fig. 2).

Occasionally a case of amyotrophic lateral sclerosis may show little demyelination in the pyramidal pathways. Two such cases were found among the other 36 cases that came to necropsy. One of these proved to be a case of spastic pseudosclerosis. The diminution of demyelination in the first 6 consecutive cases that were treated certainly could not be a mere coincidence. Furthermore, even the other 4 less adequately treated cases (Table II) showed changes which were slightly less severe than in the ordinary run of amyotrophic lateral sclerosis.

With higher magnification, the involved myelin sheaths, usually grouped in small islands, were fragmented or swollen (Fig. 3). When compared with the changes in the myelin sheaths in untreated cases, it can be stated definitely that this process was not one-tenth as extensive as in the average case of amyotrophic lateral sclerosis (Fig. 4). Occasional swelling of myelin sheaths could be detected also in parts of the pyramidal tracts which did not appear demyelinated (Fig. 5). In case 6, the right pyramidal tract was demyelinated and destruction of myelin sheaths in this pathway was more extensive.

The pathologic process in the axis cylinders, except those of the right pyramidal tract in case 6, was limited essentially to the islands of involved myelin sheaths. Here the changes, although much less intense than in the untreated cases, consisted of swelling, slight fragmentation, bulbous terminations and corkscrew appearance (Fig. 6). Various degrees of swelling of the axis cylinders were noted also in parts of the pyramidal tracts which did not appear demyelinated.

In the Sudan III preparations, the amount of fatty deposits differed in these six treated cases. There were scarcely any fatty deposits in the lateral or anterior pyramidal tracts in cases 1 and 5 (Fig. 9). Cases 2, 3 and 4 had less fat than the untreated cases, while in case 6, the fatty contents did not differ greatly from the untreated cases.

Most of the outstanding changes, however, were noted in the glial tissue. In the untreated cases of amyotrophic lateral sclerosis, there was a dense gliosis in the involved pathways easily detected with the naked eye in the Holzer preparations (Fig. 12). In the six treated cases, except for the right pyramidal tract in case 6, the gliosis could hardly be observed with the naked eye (Fig. 11). With higher magnification, however, small perivascular islands of gliosis (Fig. 13) were noted in the region of the involved myelin sheaths and axis cylinders.

In case 7 (Table II), which was treated for only a short period of time (15 days) with ephynal by mouth, the myelin sheaths, axis cylinders, fat content and glial reaction did not differ from the untreated cases of amyotrophic lateral sclerosis. In cases 8, 9 and 10 (Table II) the destruction of myelin sheaths, axis cylinders and the gliosis were not much different, although slightly less than in the untreated cases. In case 8 the fatty accumulations in the affected pathways were definitely less than in the untreated cases. The glial reaction in these cases was moderate in degree and slightly less intense than in the ordinary cases. In order, however, to be on the conservative side, the changes in these cases were considered to be about the same as those found in untreated cases.

Certain factors may have played a rôle in the histopathologic changes of cases 8, 9 and 10 (Table II). In case 8 the patient received ephynal orally, but did not receive alpha-tocopherol intramuscularly. Cases 9 and 10 were treated for 1 year and 7 months respectively, including intramuscular injections of alpha-tocopherol, but the treatment was interrupted several times. This may have influenced the lack of improvement in the involved pathways. The age, the duration of the illness and the time the treatment was begun in these instances did not differ from the other treated cases.

Factors Influencing the Changes in the Affected Pathways. As was previously demonstrated by me,¹² in untreated cases of amyotrophic lateral sclerosis the disease affects the upper and lower motor neurons. The upper motor neuron can be involved, either at its point of origin in the giant pyramidal cells of Betz or anywhere along its course. The lesion of the upper motor neuron is most pronounced in the spinal cord and is characterized by destruction of the myelin sheaths and axis cylinders, and a dense glial scar. Once the myelin sheaths and axis cylinders in the involved pathways of the central nervous system are completely destroyed, their regeneration is problematic. Regeneration of/or newly formed myelin sheaths in peripheral nerve lesions are known to occur. Recently, Tower¹³ avulsed ventral spinal nerve roots from the spinal cord in four cats that were killed after periods of from 4 weeks to 1 year. Evidence of vigorous regeneration on the part of the ventral root fibers was observed at autopsy, either grossly or on microscopic examination, in all of the animals, beginning deep in the rootlet tracts of the cord. As far as I know, this has not been demonstrated in the central nervous system. According to Cajal¹⁴ and others, the failure of regeneration in the central nervous system is due not so much to deficient power of the neurons as to the unfavorable conditions which the central tissues oppose to the growth of the newly formed

neurites. Some observers are under the impression that regeneration of axons in involved pathways of the spinal cord may take place under favorable conditions in a manner similar to regeneration of axons in peripheral nerves. By the new methods of neurofibrillar study, it has been demonstrated that the production of new fibers, clubs, cones and ramified axons may occur in various lesions of man and animals. These findings, while they demonstrate signs of repair comparable in principle with those of the central stump of the peripheral nerves, do not contradict the conception of the impossibility of complete regeneration of axis cylinders in the spinal cord. These later investigations have also demonstrated that after a certain length of time the restoration of the axon in the spinal cord stops, that the axon atrophies and that finally the nerve sprouts break down completely.

On the basis of these investigations, it can hardly be expected that completely destroyed myelin sheaths and axis cylinders in the spinal cord should regenerate. In the six cases of amyotrophic lateral sclerosis adequately treated with vitamin E there was no question that the involved myelin sheaths and axis cylinders were less affected when compared with similar preparations of untreated cases. When I¹⁵ observed similar improvement in the cases of subacute combined degeneration adequately treated with liver, I then postulated that axis cylinders may undergo a reversible reaction provided they are not severely affected and if the patient received early and adequate treatment. Can this explanation also be applied to amyotrophic lateral sclerosis? This cannot be answered as easily as in subacute combined degeneration. There is a possibility in amyotrophic lateral sclerosis, as in subacute combined degeneration, of a reversibility of the reaction if the axis cylinders are not severely destroyed. In other words, damaged but not completely destroyed axis cylinders, under favorable circumstances, may regenerate or be restored nearly to their normal state. This is the only possible explanation that can be offered from the foregoing observations. Under these circumstances, clinical improvement in the neurologic signs and symptoms in my cases should have been expected. This, as already stated, did not occur. How can this discrepancy be explained? The possibility that these patients did not receive adequate quantities of vitamin E and/or were not treated sufficiently early must be considered. In some instances of amyotrophic lateral sclerosis, in addition to the lack of vitamin E which may cause the disease, there is the possibility, as suggested by Wechsler,¹ of another unknown factor, which may play a rôle in the absorption of the vitamin. These are questions which so far have not been settled and cannot be answered with certainty. It is possible that in some instances early and

adequate administration of vitamin E may arrest the process in the involved myelin sheaths and axis cylinders and may restore their function provided they are not completely damaged.

The lessened or almost complete disappearance of fatty deposits in the treated cases can be explained on the basis that vitamin E treatment may have resulted in a decline or cessation of the pathologic process; thus helping toward the removal of the products of disintegration.

The absence of intense gliosis or the regression of the gliosis in the treated cases appears disturbing, for the contrary would be expected in areas of myelin sheath and axis cylinder repair. In a number of cases of subacute combined degeneration studied by me,¹⁵ that received adequate liver therapy, the improvement noted in the affected pathways was accompanied by a dense gliosis. There is a remote possibility that the restoration or reversion to a normal or nearly normal glial reaction as seen in the treated cases of amyotrophic lateral sclerosis is parallel to the observed restoration of the myelin sheaths and axis cylinders. If this should be true, then it may be postulated that the reversibility of reaction took place all along the line, affecting simultaneously and equally, or nearly equally, the damaged myelin sheaths, axis cylinders and glial tissue.

One other disturbing factor in this study was the change in the nerve cells in the involved bulbar nuclei and anterior horns, which did not differ in the treated and untreated cases. Clinically, however, Wechsler¹ observed that the fibrillations and the bulbar signs, especially the recent ones, cleared up, whereas, the pseudobulbar symptoms responded less well or not at all.

There are still many questions regarding the treatment of amyotrophic lateral sclerosis which will have to be answered. While the efficacy of vitamin E therapy in this disease is still problematic, the described histopathologic findings and the clinical (Wechsler¹) evidence indicate that this form of treatment should be continued experimentally.

SUMMARY AND CONCLUSIONS

Ten cases of amyotrophic lateral sclerosis were treated with vitamin E and alpha-tocopherol and, except for one, none responded clinically to this form of treatment.

Histopathologically, however, in six of the intensively treated cases the destruction of the myelin sheaths and axis cylinders was found to be much less intense than in the untreated cases. The dense gliosis which is usually present in amyotrophic lateral sclerosis was diminished or almost absent in those that received vitamin E. The lessened myelin

sheath and axis cylinder destruction and the faint gliosis in these instances were perivascular and insular in distribution. In one of these (case 6), the lessened changes were limited to one side of the cord only. The nerve cells of the involved bulbar nuclei and anterior horns remained unchanged and showed no signs of reversibility. The ultimate cause of death was bulbar in nature.

The histopathologic processes in the other four less intensively treated cases, although less extensive, were considered to be about the same as those found in untreated cases.

There is a possibility that vitamin E therapy resulted in a reversal of the reaction of degeneration affecting simultaneously and nearly equally the damaged myelin sheaths, axis cylinders and glia in amyotrophic lateral sclerosis.

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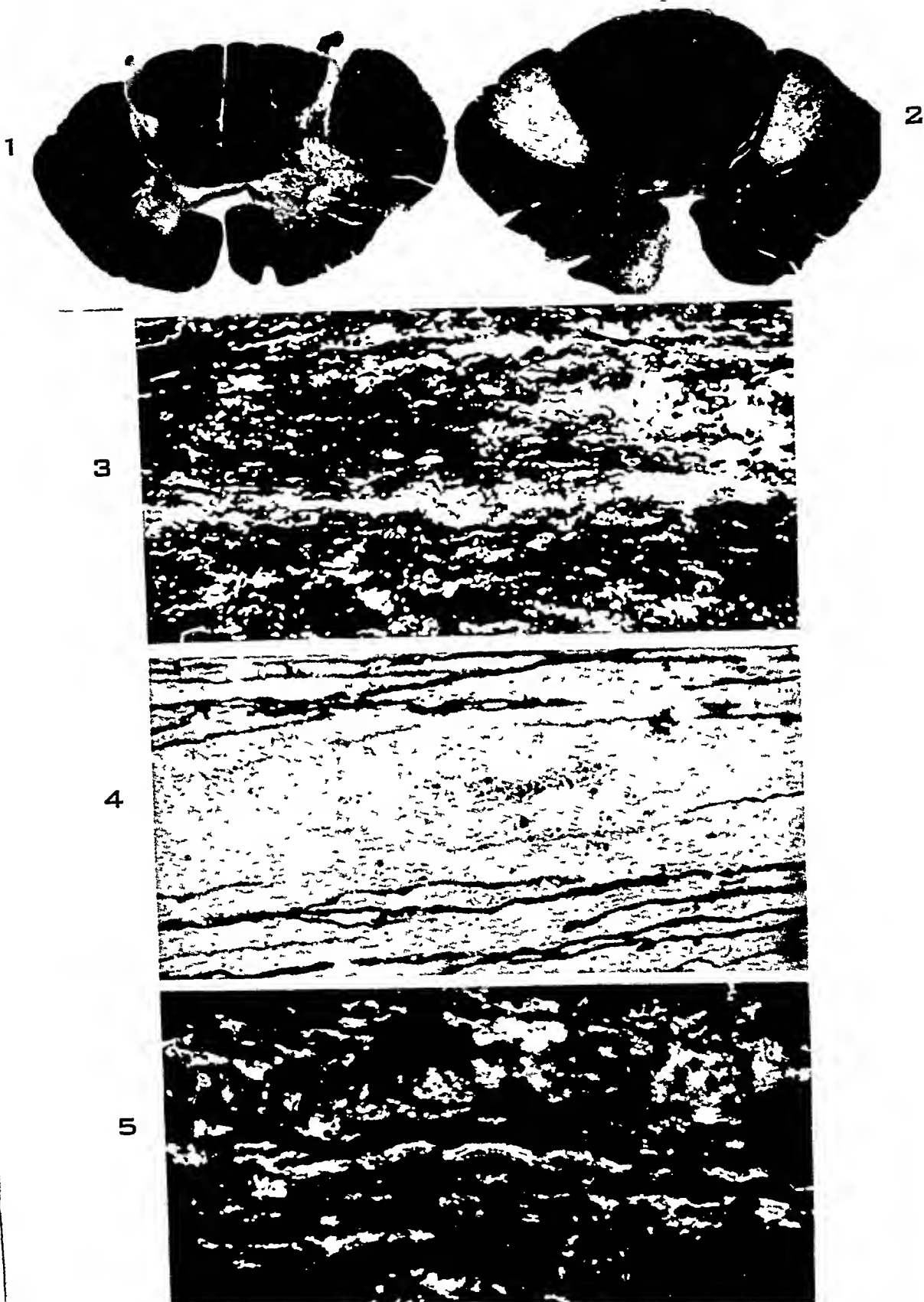
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DESCRIPTION OF PLATES

PLATE 106

- FIG. 1. Case 1. Lack of visible demyelination in the pyramidal pathways of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 2. Myelin sheath stain.
- FIG. 2. From a nontreated case, showing extensive demyelination of the crossed pyramidal and left direct pyramidal tracts. Myelin sheath stain.
- FIG. 3. Case 1. Insular myelin sheath destruction from a case treated with vitamin E. Compare with Figure 4. Myelin sheath stain. $\times 240$.
- FIG. 4. Extensive myelin sheath destruction from an untreated case. Myelin sheath stain. $\times 240$.
- FIG. 5. Case 1. Slight disintegration and swelling of single myelin fibers in parts of the pyramidal tracts that appeared uninvolved, from a case of amyotrophic lateral sclerosis that received vitamin E. Myelin sheath stain. $\times 480$.



Davison

Vitamin E in Amyotrophic Lateral Sclerosis

PLATE 107

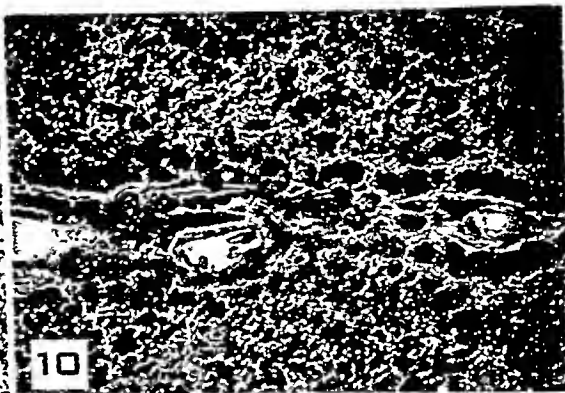
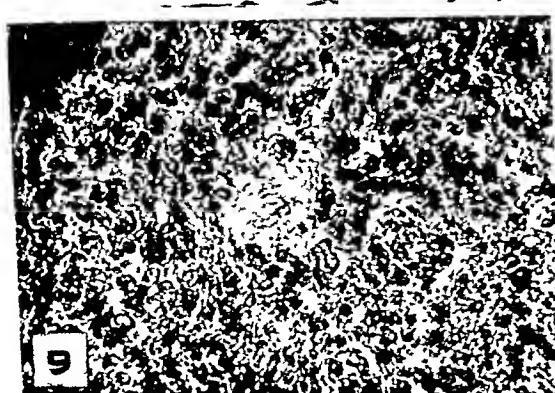
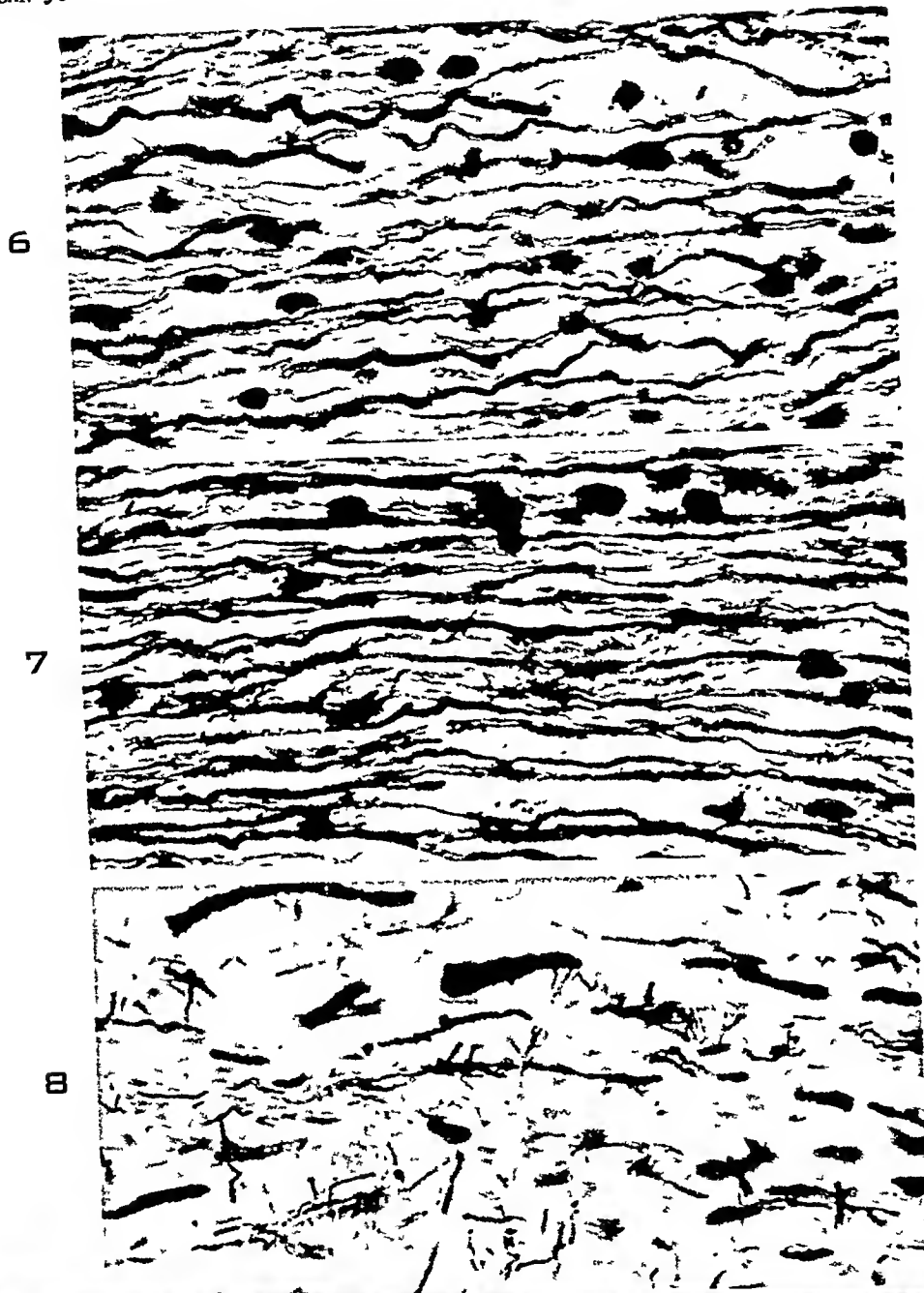
FIG. 6. Case 1. Axis cylinders from the crossed pyramidal tract of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 7 from a normal case and Figure 8 from an untreated case of amyotrophic lateral sclerosis. In Figure 6 there is slight diminution in number, swelling and slight tortuosity of axis cylinders when compared with the normal in Figure 7 and the severely diseased, fragmented and swollen axis cylinders of the untreated case of amyotrophic lateral sclerosis in Figure 8. Bielschowsky stain. $\times 480$.

FIG. 7. From a normal control case, for comparison with Figures 6 and 8. Bielschowsky stain. $\times 480$.

FIG. 8. From an untreated case of amyotrophic lateral sclerosis. For comparison with Figures 6 and 7. Bielschowsky stain. $\times 480$.

FIG. 9. Case 1. Almost complete absence of fat in the pyramidal pathways from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 10 from an untreated case showing lipoid deposits throughout and in the perivascular spaces. Sudan III stain. $\times 100$.

FIG. 10. From an untreated case of amyotrophic lateral sclerosis, for comparison with Figure 9. Sudan III stain. $\times 100$.



Davison

Vitamin E in Amyotrophic Lateral Sclerosis

PLATE 108

FIG. 11. Case 1. Lack of gliosis in the pyramidal tracts from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 12 from an untreated case of amyotrophic lateral sclerosis showing dense gliosis in the crossed pyramidal tracts. Holzer stain.

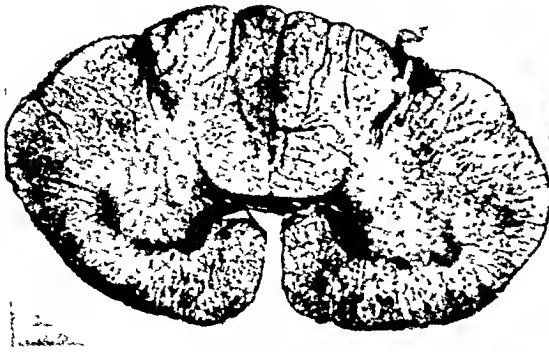
FIG. 12. From an untreated case of amyotrophic lateral sclerosis, for comparison with Figure 11. Holzer stain.

FIG. 13. Case 1. Slight insular gliosis from a treated case of amyotrophic lateral sclerosis. Compare with Figure 14 from an untreated case and Figure 15 from a case of descending degeneration. In Figures 14 and 15 the gliosis is dense; more so, however, in the case of descending demyelination. Holzer stain. $\times 200$.

FIG. 14. From an untreated case of amyotrophic lateral sclerosis, for comparison with Figure 13. Holzer stain. $\times 200$.

FIG. 15. From a case of descending degeneration, for comparison with Figures 13 and 14. Holzer stain. $\times 200$.

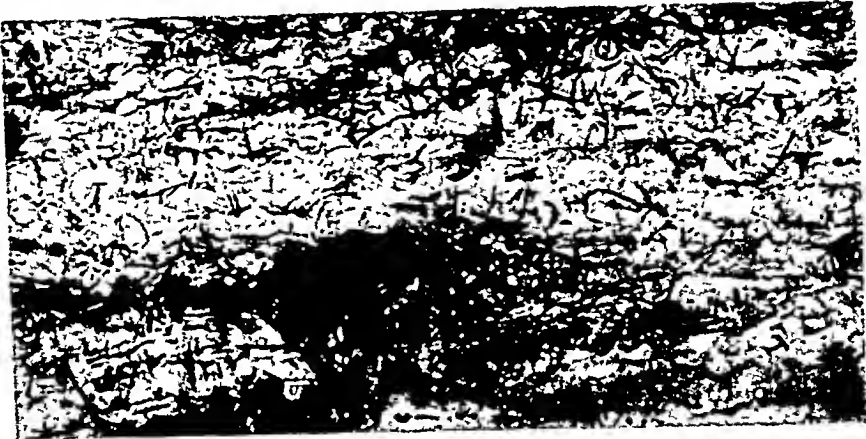
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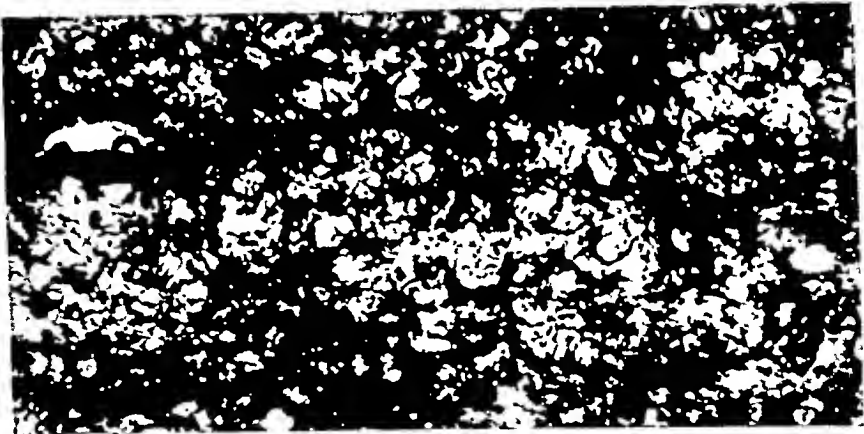
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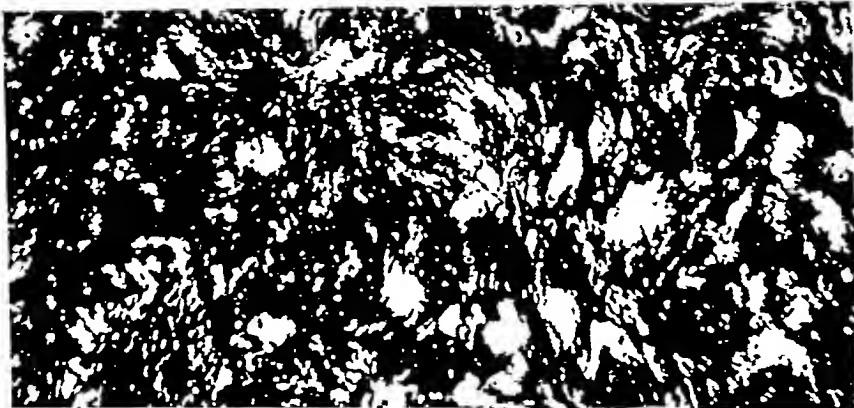
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HYPERPLASIA OF THE PULMONARY ALVEOLAR EPITHELIUM IN DISEASE*

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The pulmonary alveoli develop as saccular outgrowths of the bronchioles. In the early part of fetal life the lung has a glandular appearance and the alveoli are lined by a continuous epithelial layer. But in the latter part of the intra-uterine period the capillary bed develops extensively, and numerous capillaries break through the epithelial lining, leaving the epithelium as isolated rounded cells. A clear account of the development of the alveoli was given by Ham and Baldwin,¹ who found that they are lined chiefly by naked capillaries from late fetal life onward and that only a few rounded epithelial cells persist. In the postnatal lung occasional epithelial cells may be demonstrated in the niches between capillaries (Clara²).

It seems well established that in the postnatal lung the alveolar walls are largely bare of epithelium but that occasional epithelial cells may be found. This disappearance of the alveolar lining is presumably a functional alteration favoring a more rapid interchange of gases between the blood and the alveolar air, and it may be compared to the disappearance of the endothelial lining of the glomerular capillaries which promotes filtration through their walls.

Although lining epithelial cells are inconspicuous in the normal lung, there are numerous pulmonary diseases in which they become prominent and form a continuous epithelial layer. The various circumstances under which hyperplasia of alveolar epithelium occurs will now be described.

Chronic Passive Congestion. In long-standing chronic passive congestion due to old mitral or aortic valvular defects the lungs usually show a moderate increase of consistency throughout, and often there are firm consolidated areas suggesting pneumonia on macroscopic examination. Microscopic sections from nonconsolidated portions show a little increase in the thickness of the interalveolar septa; and a few

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epithelial cells, single or in small groups, are readily found (Fig. 1). In the consolidated areas the interalveolar septa are very thick and the alveoli are lined by a continuous layer of dark cubical epithelium (Fig. 2). The thick interalveolar septa show numerous fibrocytes and a loose edematous fibrillar connective tissue. The capillaries are reduced in number and so far from the surface that it is unlikely that any considerable interchange of gases can occur. Parker and Weiss³ described this lesion and attributed the thick septa to chronic edema. Thickening of the septa and immobilization of the tissue precede the epithelization of the alveoli. A study of transition stages shows that the new epithelial lining arises by multiplication of pre-existent alveolar epithelial cells and not as an ingrowth of bronchial epithelium.

Lipoid Pneumonia. In lipoid pneumonia the alveoli that contain fat become filled with macrophages and are largely nonfunctioning because very little air can gain entrance. In such alveoli several observers have noted epithelization. The alveolar epithelium may be present in patches or it may form a continuous layer (Fig. 3). A study of transition stages indicates hyperplasia of persistent alveolar epithelium as in chronic passive congestion. The epithelial cells are often desquamated, but they apparently do not act as phagocytes. As in chronic passive congestion, epithelization is preceded by thickening of the interalveolar septa and loss of function.

Chronic Interstitial Pneumonia. In unresolved pneumonia there are often areas of chronic interstitial pneumonia, and in such lesions there are frequently foci with marked epithelization of the alveoli. Oberndorfer⁴ described such changes in a case of hemorrhagic pneumonia.

As an example of epithelization in chronic interstitial pneumonia the following case is cited: A man, 67 years old, died of pneumonia of unknown duration. The pathologist, Dr. J. F. Noble, found large irregular areas of consolidation of fibrous consistency in each lung. Upon microscopic examination the consolidated areas were found to be chiefly due to interstitial pneumonia. Within and around the solidified areas many of the alveoli were lined by cubical or columnar epithelium, giving the tissue a glandular appearance (Fig. 4). Some of the columnar cells were filled with mucin but no ciliated cells were seen. The presence of cylindrical cells filled with mucin suggests that bronchial epithelium has grown into the alveoli, but numerous transition stages indicate that the epithelium develops from small cells that were already present on the alveolar wall. The first stage in epithelization is the formation of a discontinuous epithelial layer (Fig. 5), which then increases to form a solid cubical or columnar layer. Many of the epithelial cells are desquamated into the alveoli.

Grumbach⁵ studied chronic interstitial pneumonia in guinea-pigs, which he attributed to injections of a diphtheroid bacillus obtained from a lymph node of a patient with Hodgkin's disease. One may doubt whether the pneumonia was due to the organism injected, but a diffuse alveolar epithelization developed which Cowdry⁶ considered similar to that in the lungs of jaagsiekte in sheep.

Epithelization of the alveoli is of common occurrence in diseased lungs. It is often found adjacent to the walls of old empyema cavities and old pleuritic adhesions as well as in all forms of interstitial pneumonia. The basic disturbance responsible for the formation of alveolar epithelium seems to be a loss of respiratory function due to thickening of the interalveolar septa or filling of the alveoli with foreign material.

Tar and Tar Derivatives. Simonds and Curtis⁷ injected tar, dissolved in heavy liquid petrolatum, intravenously into rabbits, and obtained marked alveolar epithelization about necrotic areas in the lungs.

Grady and Stewart⁸ induced pulmonary tumors in strain A mice by subcutaneous injection of 1:2:5:6-dibenzanthracene or methylcholanthrene. Multiple tumors began to develop about 5 weeks after the injection. The tumors were all of alveolar origin, and they began as a layer of cells lining the alveoli. The lining epithelium then continued to grow to form a carcinoma. The authors pointed out the similarity of the lesion to jaagsiekte in sheep.

Jaagsiekte. Jaagsiekte is a serious endemic infectious disease in sheep prevalent in South Africa. A similar disease in sheep in Iceland was described by Dungal.⁹ Among the prominent symptoms are dyspnea, easy fatigability and a watery discharge from the lungs. The lungs are extensively consolidated. Cowdry and Marsh¹⁰ have described the histologic changes. The alveoli are all lined by cubical or columnar epithelium, giving the lung a glandular appearance. The structure suggests carcinoma, but there are no metastases and the interalveolar septa are intact. The etiology of jaagsiekte is unknown, but its infectious nature and the peculiar proliferative reaction limited to alveolar epithelium suggests a virus.

DIFFUSE EPITHELIAL HYPERPLASIA IN MAN

Bonne,¹¹ in 1939, described an unusual pulmonary lesion in a Chinese male, 30 years of age. The duration of the disease was about 9 months. It began with a fever of about 4 weeks' duration followed by a cough which persisted. The sputum contained mucus but no blood. In the later months there was dyspnea and emaciation, and roentgenograms showed a massive consolidation of the left lung. The blood picture was normal. At autopsy the lungs weighed 2700 gm.,

and were almost completely solid and homogeneous. Only the right apex was uninvolved. The appearance suggested lobar pneumonia with hepatization but the lungs were a little firmer. Microscopically all of the alveoli were lined by cubical or columnar epithelium. Bonne at first considered the growth to be a carcinoma but, since there were no metastases and the interalveolar septa were intact, he finally interpreted it as adenomatosis. He pointed out the striking resemblance to jaagsiekte in sheep.

Report of Case

A case similar to Bonne's¹¹ came under my observation. The patient, a male, 63 years of age, was admitted to the University Hospital on March 5, 1942, on the service of Dr. C. J. Watson, complaining of dyspnea on exertion, productive cough, weakness and loss of weight. His illness began about 2 years before admission with these symptoms. He had dyspnea on slight exertion, and he produced an abundant, clear, frothy sputum. He had lost about 25 lbs. He had no fever, chills or night sweats. Hemoglobin was 14 gm. per cent; leukocytes, 12,000, with 72 per cent neutrophils and 24 per cent lymphocytes. The urine was normal. The Wassermann reaction was negative. Physical examination revealed consolidation of the left lower and right middle lobes. After introduction of lipiodol the small bronchi were of unusual appearance in that they showed a diffuse narrowing. The tentative clinical diagnosis was carcinoma of the lung.

The patient was finally dismissed from the hospital, and he died at home on November 16, 1942. The family physician removed parts of the lungs and sent them to the University. He made no observations as to metastases.

The specimens submitted were the consolidated lobes of each lung. These had the appearance of lobar pneumonia in that there was a diffuse consolidation. Microscopic examination showed changes entirely similar to those described by Bonne.¹¹ Every alveolus was lined by cubical or columnar epithelium (Figs. 6 and 7). The interalveolar septa were moderately increased in thickness but this was due chiefly to the epithelial layers and not to leukocytic infiltration and fibrosis as in interstitial pneumonia. The septa were never destroyed by epithelial invasion as one would expect if the growth were a carcinoma.

The lesion is clearly not an interstitial pneumonia, and epithelization of the alveoli is not due to thickening of the septa or to foreign material in the alveoli. There is presumably some direct stimulation of the alveolar lining cells. The epithelial layer separates the capillaries from the alveolar air and greatly impedes the interchange of gases. This is presumably the cause of the dyspnea.

ADENOMAS AND CARCINOMAS ARISING FROM THE ALVEOLAR EPITHELIUM

There is a widespread opinion that all carcinomas of the lungs arise from the bronchi; yet there are a few reported cases with strong evidence of an alveolar origin.

Helly,¹² in 1907, described multiple adenomas of the lung in a woman, 43 years old. Richardson,¹³ in 1940, reported numerous firm nodules, 2 to 10 mm. in diameter, in the lungs of a woman, 73 years old. The tumors were sharply circumscribed and did not connect with bronchi. Microscopically the growths were composed of closely set alveoli lined by tall columnar epithelium which formed mucin. The interpretation was multiple adenomas.

Weissmann,¹⁴ in 1935, described two instances of alveolar tumors. (a) A male, 73 years old, with numerous gray nodules in both lower lobes. The interalveolar septa appeared to be normal and were lined by tall columnar epithelium. (b) A woman, 65 years old, showed numerous nodules throughout both lungs with metastases in the bronchial nodes. Microscopically the alveolar structure was retained and the alveoli were lined by tall columnar epithelium.

Sweany,¹⁵ in 1935, described a lung from a woman, 67 years old, which had the macroscopic appearance of lobar pneumonia. Microscopically there was an alveolar structure with alveoli lined by a single layer of epithelium. There was a metastasis in one bronchial lymph node.

Neubuerger,¹⁶ in 1941, published the report of a male patient, 39 years of age, with multiple yellowish nodules, 2 to 10 mm. in diameter, throughout both lungs. There were metastases in the mediastinal and periaortic lymph nodes, the liver and the kidneys. Microscopically the alveoli were lined by tumor cells but the interalveolar septa were intact.

The human pulmonary tumors reported as multiple adenoma or carcinoma have a microscopic structure similar to that of my case and the one reported by Bonne.¹¹ The alveoli are lined by epithelial cells and the interalveolar septa are intact. In a few instances metastases have been found. There is no good reason to doubt that hyperplasia of the alveolar epithelium may give rise to localized or diffuse adenomatous growths which may form metastases.

DISCUSSION

In the postnatal lung the alveolar walls are formed almost entirely by the capillaries, but a few epithelial cells persist in the niches between capillaries. In any disease which brings about marked thickening of the interalveolar septa, with displacement of the capillaries away from the surface and consequent loss of respiratory function, the alveolar epithelium may undergo hyperplasia to form a continuous epithelial lining. The cells are either cubical or columnar and some of them may secrete mucin. There is convincing evidence that the

epithelium forms locally and does not grow in from the bronchi. In chronic passive congestion and in interstitial pneumonia, epithelization of the alveoli follows thickening of the interalveolar septa.

Mild irritation of the alveolar walls from foreign bodies may bring about epithelization, as in lipid pneumonia.

In jaagsiekte of sheep widespread epithelization of the alveoli occurs. In this infectious disease the etiology is unknown but the microscopic features suggest direct irritation of the cells by a virus which attacks them.

In man there are now two examples of widespread epithelization similar to jaagsiekte in sheep (Bonne,¹¹ Bell). These may be regarded as diffuse epithelial hyperplasia due to an unknown irritant. In the absence of metastases and destructive infiltrative growth, the process in these cases can hardly be interpreted as neoplasm, but Sweany's¹⁵ report suggests a neoplastic nature.

SUMMARY

A case of extensive diffuse epithelization of the alveoli of the human lungs is reported which is similar to the one reported by Bonne.¹¹

Epithelization of the alveoli is often seen in chronic passive congestion and interstitial pneumonia, apparently as a result of thickening of the interalveolar septa.

Foreign material in the alveoli also causes epithelization, as in lipid pneumonia.

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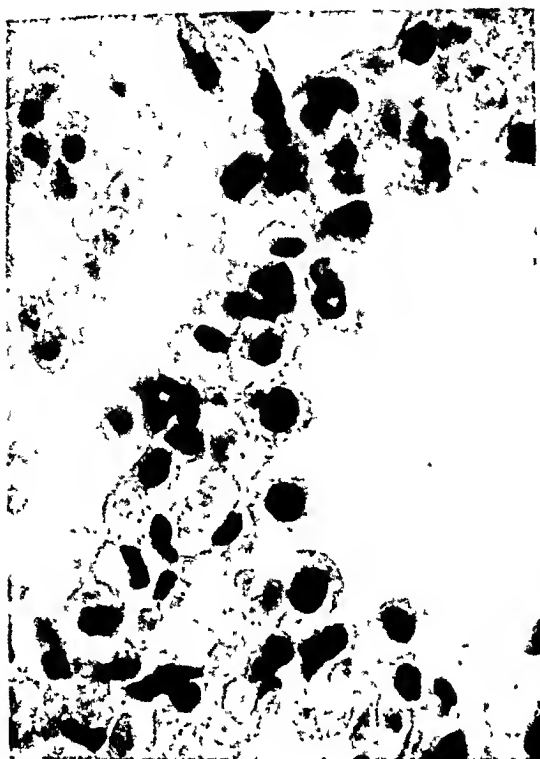
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[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 109

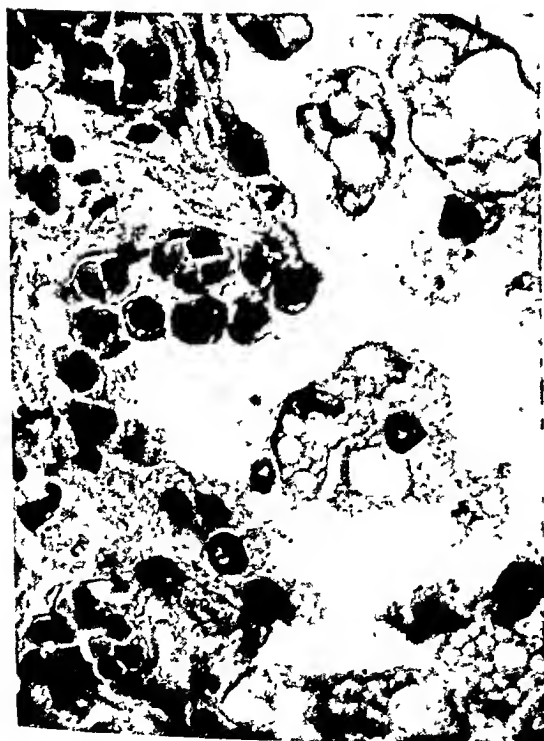
- FIG. 1. Chronic passive congestion from mitral stenosis and aortic insufficiency. A nonconsolidated area of the lung shows several rounded alveolar epithelial cells on the right side of the interalveolar septum. The greater part of the septal walls is bare. Hematoxylin and eosin stain. $\times 350$.
- FIG. 2. Chronic passive congestion from an old rheumatic defect of the aortic valve. Thick interalveolar septum from a consolidated area. The alveoli are lined by dark cubical epithelium, and the few small capillaries are at some distance from the surface. Hematoxylin and eosin stain. $\times 250$.
- FIG. 3. Lipoid pneumonia showing macrophages filled with fat in the alveoli, and a layer of alveolar epithelial cells on the wall of the septum. Hematoxylin and eosin stain. $\times 250$.
- FIG. 4. Chronic interstitial pneumonia. The persisting alveoli are lined chiefly by tall columnar cells filled with mucin. Hematoxylin and eosin stain. $\times 80$.



1



2



3



4

Bell

Hyperplasia of Pulmonary Alveolar Epithelium

PLATE 110

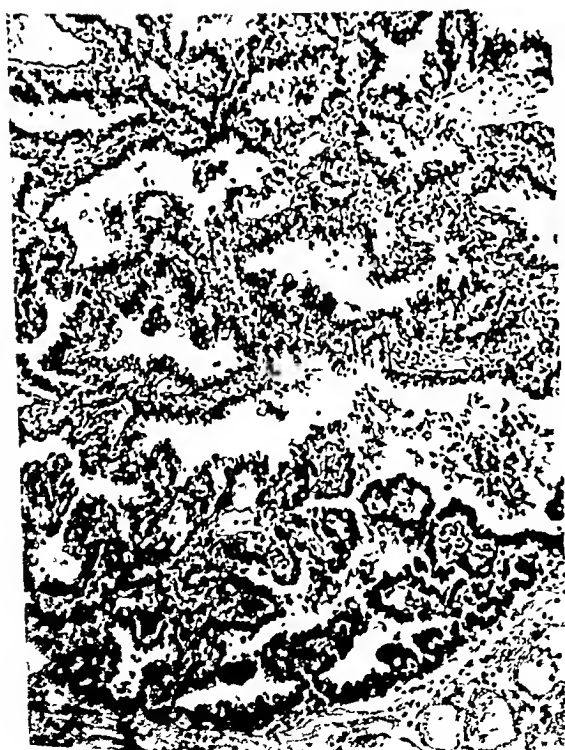
FIG. 5. Chronic interstitial pneumonia. From the same lung as Figure 4. The alveolar epithelium forms a thin discontinuous layer on the upper right side of the septum and a solid epithelial layer in other places. Some desquamated epithelial cells are seen in the alveoli. Hematoxylin and eosin stain. $\times 250$.

FIG. 6. Area of consolidated lung from the author's case. The alveoli are all lined by cubical or columnar epithelium. Hematoxylin and eosin stain. $\times 60$.

FIG. 7. Same area as in Figure 6 under higher magnification. The thickening of the septa is due chiefly to the epithelial layers. Hematoxylin and eosin stain. $\times 100$.



5



6

Bell



7

Hyperplasia of Pulmonary Alveolar Epithelium

THE PULMONARY ALVEOLAR LINING UNDER VARIOUS PATHOLOGIC CONDITIONS IN MAN AND ANIMALS *

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The presence of lining cells in the pulmonary alveoli in various pathologic conditions has often been mentioned in papers and textbooks. In addition, proliferation of these alveolar lining cells has been produced experimentally. To our knowledge, however, El Gazayerli ¹ is the only author who ever attempted to collect and describe such pathologic material systematically. This lack of organized studies perhaps explains the absence of a special chapter on this subject in most modern American textbooks of pathology. Bell ² alone discusses the matter briefly. Contemporary pathologists (Bell, ² Boyd, ³ Karsner, ⁴ MacCallum, ⁵ Smith and Gault, ⁶ Delafield and Prudden ⁷) apparently accept the presence normally of alveolar epithelium, which proliferates under various stimulations. On the other hand, anatomists (Maximow and Bloom, ⁸ Cowdry, ⁹ Miller ¹⁰) disagree as to the structure of the alveolar wall and as to the existence of an alveolar lining. Anatomists, however, do not treat thoroughly the subject of alveolar cell proliferation because it is a phenomenon found in pathologic conditions. Our interest in the subject became aroused during an investigation of lung tumors believed to take origin from the pulmonary alveoli. As a result, we have made a study of the alveolar changes found in human lungs under various pathologic conditions and the alterations in animal lungs resulting from spontaneous diseases.

THE NORMAL PULMONARY ALVEOLUS IN MAN AND ANIMALS

Human embryologic studies suggest that the epithelial lining of the terminal vesicles begins to disappear about the end of the fifth month of fetal life (Barnard and Day, ¹¹ Palmer, ¹² and others); prior to that time, the lung has a distinct tubular or glandular appearance. Policard ¹³ pointed out that as late as the sixth month the lung is really a bronchiolar structure in a mesenchymal stroma; no true alveoli are present before that time and those that develop thereafter are not lined by epithelium. A different view was expressed by Bensley and Groff, ¹⁴ Zeldes, ¹⁵ and others, who believed that merely a transition from cuboidal to flattened epithelium occurs in the alveoli during fetal life.

Until respiration takes place, the extent and character of the alveoli

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are poorly defined microscopically, because the potential air spaces are not expanded. In the lungs of newborn infants, who have died soon after birth from nonrespiratory causes, most of the alveolar walls are somewhat thick and cellular. They are composed of capillaries with a typical endothelial lining. Frequently a fairly characteristic cell, probably mesenchymal in origin and distinguishable with difficulty in the normal adult lung, is found in the alveolar wall and in the interstices between the capillaries. This cell resembles the endothelial cell in that its nucleus is pale, vesicular, fairly large, and usually oval; it differs from the endothelial cell in that it is less definitely related to a capillary and has an abundant clear or very slightly granular cytoplasm, which is irregular in contour. This is the so-called "septal cell," a term introduced into the American literature by Lang¹⁶ in 1925. This term corresponds to the word "epicyte," introduced by Clara¹⁷ and applied by Macklin,¹⁸ and to the designation "alveolar cell," commonly used for tumors believed to arise from these cells, namely, "alveolar cell tumor" or "alveolar cell carcinoma." According to the opinion expressed by Maximow and Bloom,⁸ Lang,¹⁶ Fried,¹⁹ Loosli,²⁰ and others, there is no epithelial lining in the alveoli during normal postnatal life. This is contradictory to the views of Miller,¹⁰ Bremer,²¹ Cooper,²² and others, who believed that a continuous epithelial lining exists, and to those of Aschoff,²³ Bargmann,²⁴ Seemann,²⁵ and others, who thought that the isolated septal cells in the capillary niches are epithelial. Clara¹⁷ and Macklin¹⁸ stressed the origin of these cells from entodermal epithelium. These various divergent opinions were clearly summarized at a round table conference conducted by Macklin.²⁶

The epithelium of the respiratory tract appears to terminate more or less abruptly at the beginning of the alveolar ducts. The walls of both the alveoli and ducts are composed of capillaries in a delicate reticular and elastic stroma. The septal cells scattered therein are usually distinguished easily from the bronchiolar epithelium.

The normal lung structure in animals, as observed chiefly in cattle, sheep and hogs, and to a less extent in dogs and cats, is similar to that in human beings—the alveoli and ducts consist of capillaries in a delicate reticulo-elastic stroma. Although isolated septal cells are present, no definite alveolar lining is evident in the lungs from healthy mature animals.

THE HUMAN ALVEOLAR LINING UNDER PATHOLOGIC CONDITIONS

Introduction

The human material was selected from a series of 4,000 necropsies, which had been performed at the Colorado General Hospital. The

descriptions are based on paraffin-embedded lung sections, 6 μ thick, stained by the hematoxylin and eosin method. In certain instances, thicker sections, to a maximum of 25 μ , were stained for elastic and reticulum fibers, fat droplets, pigment, and glycogen. We are of the opinion that careful study of such routinely prepared lung tissue offers abundant information on this subject.

The changes to be described were observed in lungs with pneumonias, infectious granulomas, neoplasms, and miscellaneous conditions. Some idea of the frequency of these changes for each condition will be given, but no actual statistics can be furnished. The number of specimens of certain diseases was too small to be of value statistically. Our findings are based on sampling routine material rather than on exhaustive analysis of such disease. We feel that such sampling, even though incomplete, provides a fairly accurate impression as to the frequency of the changes. The terms "uncommon," "fairly common" and "frequent" will be used to denote the comparative frequencies. It is hoped that this attempt to correlate the fundamental facts and observations as to septal cell proliferation will stimulate further investigation.

Pneumonias

Bacterial. In textbooks on pathology (Boyd,³ Karsner,⁴ MacCallum,⁵ Aschoff,²⁷ Lauche²⁸) mononuclear phagocytic proliferation is described in the stage of resolution of lobar pneumonia. Some authors felt that these phagocytes are really desquamated epithelium, but placed no emphasis on distinct lining-formation in this disease. In our experience, septal cell proliferation and the formation of a definite lining are not observed in red or gray hepatization *per se*. They may occur in any stage, however, if the pneumonia is accompanied by other conditions. Figure 1 is an example of such proliferation in red hepatization, associated with chronic passive congestion of the lungs secondary to subacute bacterial endocarditis. Fibrin, erythrocytes, polymorphonuclear leukocytes, and mononuclear phagocytes are present in the lumina of the alveoli; the alveolar walls exhibit distinct but incomplete lining by low cuboidal cells. In the later stages of lobar pneumonia, especially when it is complicated by delayed resolution and organization, lining formation may readily occur. Figure 2 shows this feature in a chronic organizing pneumonia. In bronchopneumonia of bacterial origin, lining is uncommon during the early stages just as in the lobar form, but it may occur during delayed resolution and organization. Scattered septal cell swelling, however, may be observed in any stage (Fig. 3).

Viral. In pneumonia caused by viruses, proliferative characteristics

of the septal cells have been described repeatedly. MacCallum²⁹ reported such reactions in measles during World War I. In influenzal pneumonia desquamative and proliferative characteristics of the septal cells have been observed. However, in these pneumonias it is impossible to exclude the part played by complicating bacteria. Güthert³⁰ reported proliferative reactions in the septal cells in the pneumonia of psittacosis, as also noted recently by Appelbaum and Ackermann.³¹ Feyrter³² described septal cell proliferation in the pneumonia complicating pertussis; and Goodpasture, Auerbach, Swanson and Cotter,³³ though not stressing this feature, illustrated it in their photomicrographs of pneumonia in pertussis and measles. We³⁴ reported septal cell swelling, proliferation, and lining in pneumonia in chickenpox. Figures 4 and 5 demonstrate these features in pertussis and chickenpox, respectively. In summary, septal cell proliferation and alveolar lining are frequently observed in pneumonias associated with virus infections.

Chemical. Lipoid pneumonia, due to aspiration of oily substances, has received much attention in recent years (Goodwin,³⁵ Gowar and Gilmour,³⁶ Graef,³⁷ Ikeda³⁸). The changes are of mixed acute and chronic character. Whereas Goodwin interpreted the cellular proliferation as arising from the "alveolar epithelium," Graef and Ikeda believed that it resulted from downgrowth of bronchiolar epithelium; Gowar and Gilmour, on the other hand, traced it to fixed histiocytes of the alveolar walls. In Figure 6 we offer an example of lipoid pneumonia showing prominent cuboidal-cell linings in the alveoli. In this instance there was no evidence of bronchiolar participation. Delafield and Prudden⁷ described and illustrated pulmonary changes following nitric oxide poisoning in which the alveoli displayed similar lining.

Aspiration Other Than Lipoid. Good examples of septal cell proliferation in nonlipoid aspiration were not observed. This may be related to the factor of time, since such patients rarely live long enough following the development of aspiration pneumonitis to produce septal cell growth.

War Gases. We have had no experience with this type of inflammation and were unable to find reports in connection with the current war. Groll³⁹ stated in this regard that the "alveolar epithelium" became enlarged and formed a prominent lining in the bronchopneumonia due to phosgene poisoning in World War I.

Physical Agents: X-Rays and Radium. Warren and Gates⁴⁰ studied the pulmonary response to these agents in experimental animals and correlated the changes with those observed in man. They examined four species, the dog, pig, rabbit and rat, and noted various stages

of irradiation reaction up to a degree fairly constantly seen in man. Rupture and reduplication of the elastic framework, a hyaline lining membrane, and epithelial anaplasia of both bronchogenic and alveolar origin were found. Similar observations were made by Bauer and Schraer.⁴¹ We had one example in our series, that of a woman, 40 years of age, to whom irradiation therapy had been administered because of a bronchial polyp that had undergone malignant change. Figure 7 illustrates the septal cell proliferation and alveolar lining, mobilization of foamy macrophages in the alveolar lumina, edema, and interstitial fibrosis in this case.

Infectious Granulomas

Although septal cell proliferation occurs not infrequently in acute inflammatory disease, it is much more common in specific chronic inflammations. The changes have been observed with almost all types of granulomas, but we shall restrict ourselves to a description of them as found in the more important granulomas.*

Tuberculosis. Active tuberculous foci commonly exhibit perifocal septal cell proliferation and alveolar lining formation. Several authors have described this (El Gazayerli,¹ Karsner,⁴ Pagel and Henke,⁴³ and others). Illustrations are shown in Figures 8 and 9. Figure 8 displays lining formation in and around typical tuberculous granulation tissue; the alveoli are distorted; some of the lined spaces are probably alveolar ducts. Figure 9 demonstrates acute tuberculous exudation with many foamy macrophages in the lumina and distinct ribbonlike cuboidal cell lining.

It is well known that in and around apical scars cuboidal cells frequently line the open spaces. The question whether all such scars are of tuberculous origin is unsettled and we prefer not to discuss it here. Figure 10 shows a large area of apical fibrosis, in which centrally located spaces or distorted alveoli are lined by cuboidal cells, most of which appear to be firmly attached to the wall. Lining formation is apparent also along the margins of the scar.

Syphilis. Pulmonary involvement by this disease is extremely rare, at least in this country. We have found only one case showing pulmonary gummas. In that instance there was moderate perifocal septal cell proliferation and lining, essentially similar to the formation around tuberculous granulomas. Wohlwill⁴⁴ has recently compiled an extensive series of cases of pulmonary syphilis from material in Portugal.

* Interstitial pneumonia caused by toxoplasmosis was reported recently by Paige, Cowen and Wolf.⁴² In their report septal cell proliferation was described. We have had no experience with this disease.

He described and illustrated alveolar lining within areas of luetic fibrosis. In pneumonia alba of congenital syphilis such lining, in addition to interstitial thickening, is generally observed.*

Neoplasms

Primary. The question whether true tumors may arise from alveolar lining cells is not settled at the present time. Many authors believe that all cancers of the lung are bronchogenic in origin. Others, on the basis of alveolar cell studies under various influences, have advanced the possibility "that conditions might arise which would make of this cell the nucleus of a primary cancer of the lung" (Macklin¹⁸). In a recent general review we⁴⁵ summarized the evidence in support of the existence of a specific tumor that arises from the septal cells, lines the alveolar walls, and is entirely independent of the bronchial system. The tumor may be found in a multinodular or in a diffusely infiltrating form. We preferred the designation "alveolar cell tumor," because this term leaves open the question of the nature of these alveolar cells; the term "carcinoma" would ascribe the origin to entodermal epithelium. Twenty-five instances of malignant and 4 of benign tumors of this type were compiled from the literature; in 15 additional cases, most of which were definitely malignant tumors, alveolar origin was possible if not probable. The case of Smith and Gault,⁶ to which our attention has been called recently, should be added to the malignant group. Our own material included 5 additional examples of the latter form and we think that the alveolar cell tumor is not as rare as heretofore believed. The histologic features have been described at length in the review previously mentioned; suffice it to say here that the alveoli are lined by cuboidal or columnar neoplastic cells with papillary protrusions. Figure 11 shows a neoplastic lining; some of the cells exhibit bizarre forms. Close relation to intra-alveolar cells of phagocytic character is apparent. The section is from a multinodular type of alveolar cell tumor in a male, 39 years old (reported by one of us⁴⁶ in 1941).

Proliferation of lining cells in inflammatory conditions to a degree suggesting benign neoplasm occurs rarely in man (Oberndorfer⁴⁷) but much more frequently in animals. This will be discussed later.

Metastatic. Tumors involving the lungs from foci elsewhere are occasionally surrounded by a perifocal septal cell reaction. This is to be distinguished from the actual lining of alveoli by neoplastic cells, a feature observed now and then in neoplasms which metastasize to

* An excellent illustration is furnished in Figure 423, p. 745, of the 7th edition of MacCallum's textbook.⁵

the lungs (Stahr,⁴⁸ Klotz⁴⁹) and in bronchogenic tumors, which utilize the alveolar walls for a framework.

Miscellaneous Conditions

Silicosis. Septal cell proliferation in the alveoli surrounding silicotic nodules is commonly found and has been described by other workers. The alveoli bordering small discrete foci are frequently distorted and stretched as a result of the contraction of the nodule. In such cases the lining may be incomplete and only the wall directly adjacent to the focus may show an epithelioid, ribbonlike covering. Massive conglomerate silicosis rarely exhibits intrafocal lined spaces; cuboidal cells, however, are apparent in the alveoli adjoining the larger scars. Our material comprised about 50 cases of this disease. An example is offered in Figure 12.

Vascular Disturbances. In chronic passive congestion of the lung, septal cell swelling is frequently observed. True lining formation is uncommon, although noted by us in a few instances. In Figure 13 the alveolar lumina are filled with pigmented macrophages and the walls are bordered by cuboidal cells.

The alveoli around pulmonary infarcts occasionally exhibit septal cell proliferation and lining formation, particularly after supervening inflammation and fibrosis. Fischer⁵⁰ described this thoroughly in 1922. If the infarct is acute and death occurs rapidly, septal cell proliferation may not be found.

Scars of Undetermined Origin. The presence of a cellular lining in and around pulmonary scars is fairly common and is similar to that observed in tuberculosis and silicosis. Within larger scars one cannot be certain whether the lined spaces are really distorted alveoli, terminal bronchioles, or tissue clefts. The latter possibility is an interesting one. We have been unable to find a discussion of this question in the literature. The tendency of mesenchymal cells to form a continuous lining of open spaces is well demonstrated in certain tumors, notably synoviomias (Black⁵¹).

Atelectasis. In acute pulmonary collapse, septal cell swelling may be marked, but true lining formation is usually absent. Chronic atelectasis and fibrosis are often accompanied by septal cell proliferation and lining formation.

Pulmonary Inflammation in the Newborn. The alveolar walls in the newborn appear more cellular and thicker than in later life, as previously mentioned. The lumina are relatively smaller and the septal cells are more prominent. However, a distinct and complete alveolar lining does not form commonly under the influence of acute inflammation.

Pleurisy and Empyema. A definitely increased tendency toward proliferation of the septal cells of alveoli bordering the visceral pleura has long been recognized. This occurs under various conditions. No satisfactory explanation has been advanced and there is no evidence of penetration by the surface mesothelium. In pleural inflammatory diseases lining is frequently seen in the subjacent alveoli (Fig. 14).

Lung Abscess and Gangrene. Perifocal septal cell proliferation and lining are not infrequent in association with abscess and gangrene of the lung, especially in the more chronic forms.

PULMONARY ALVEOLAR LINING IN ANIMALS UNDER PATHOLOGIC CONDITIONS

Acute Inflammation

In the older literature Bosc⁵² and Borrel⁵³ pointed out the existence of "alveolar epithelial" proliferation in sheep pox; more recently Thorp and Hallman⁵⁴ reported a similar reaction in pneumonia in calves. We have examined 25 specimens of spontaneous acute pneumonia in different species, such as the hog, lamb, fox, goat, cat, horse and cow. An unusual opportunity was afforded by an instance of pneumonia in a tiger. Several cases of pneumonia in turkeys also were examined. The etiologic agents varied. In the early phases of pneumonia, septal cell swelling was commonly observed. Actual lining, however, could be seen in only a few instances of acute pneumonia.

In experimental work in animals various agents have been used successfully to produce acute inflammation with proliferative reactions of the septal cells. Among the etiologic factors, bacteria, viruses, toxins and chemicals may be mentioned. The exudate in such experiments frequently shows a predominance of mononuclear cells. We discussed this subject to some extent in a previous report³⁴ and, for comprehensive details, refer the interested reader to the paper by Ross.⁵⁵

Chronic Inflammation

The total material consisted of 26 cases. One of us (C. L. D.) has observed at least 1,000 examples of chronic pneumonia in sheep on the slaughter floor. Together we have studied 20 such cases grossly and microscopically. The other chronic infections in our series were in the cow, goat and hog.

Chronic Progressive Pneumonia in Sheep. This is the condition that has been variously designated as "jagziekte," "Montana chronic progressive pneumonia" and "Icelandic adenomatosis." Excellent descriptions have been given by Bonne,⁵⁶ Cowdry and Marsh⁵⁷ and Dungal.⁵⁸

The disease is progressive over many months, sometimes over a year or more. Since many pathologists are unfamiliar with the condition, we shall describe the changes briefly. The lungs are heavy and on section display many solid, gray, tumorlike foci, which vary in size and shape and may involve almost the entire organ. The intervening tissue sometimes exhibits bronchopneumonia. Histologically, the picture is striking in its similarity to adenoma or even carcinoma. Cowdry and Marsh, and Dungal stated that several pathologists to whom they submitted sections made a diagnosis of carcinoma. In the tumorlike foci both alveolar and bronchogenic cellular proliferation occurs. Figure 15 shows alveoli and alveolar ducts, lined by cuboidal cells, which form occasional papillary projections into the lumina. The alveolar septa are thickened and infiltrated by chronic inflammatory cells.

Chronic Pneumonias and Granulomas in Other Species. Chronic pneumonia continuing over many months, as observed in sheep, is infrequently seen in other species. In our collection we have examined 6 cases—3 in cattle, 2 in goats and 1 in a hog. Theiler⁵⁹ described a related condition in horses. Olafson and Monlux⁶⁰ recently reported similar features in toxoplasmic pulmonary infection in a cat, and Gruber⁶¹ observed them in verminous pneumonia in deer. In such instances the alveolar septal cell and bronchogenic proliferation closely simulate the histologic changes described in the chronic progressive pneumonia in sheep. We have noted perifocal septal cell proliferation also in animals of various species that had suffered from such rare chronic infections as actinobacillosis, actinomycosis, aspergillosis, coccidioidal granuloma, distomiasis and verminous pneumonia. Intrafocal lining is seen sometimes in the more fibrosed areas of these inflammatory conditions but far less frequently than in the chronic progressive pneumonia of sheep.

Neoplasms

Although we have personally observed several cases of alveolar cell tumor in man and have looked for its counterpart in animals, particularly in sheep and cattle, we have been unable to find satisfactory examples. The septal cell proliferations in the chronic progressive pneumonia of sheep are not considered true neoplasms by most authorities in this field. To us this interpretation seems correct. In other species, however, namely, mice, tumors that arise from the alveolar walls either spontaneously or experimentally have been described (Wells, Slye and Holmes,⁶² McDonald and Woodhouse⁶³). Grady and Stewart⁶⁴ experimentally produced tumors in strain A mice by subcutaneous injections of methylcholanthrene and of dibenzanthracene.

The tumors were of multicentric origin and the histogenesis was described as follows in the abstract which they submitted:⁶⁵

"Immediately preceding the development of tumors and accompanying the early stages of their formation was a notable proliferation of large mononuclear cells from the alveolar walls. . . . Frequently they would form columns of various length partly or completely lining the alveolar space, or they might coalesce to form small groups. These groups might project into the alveolar lumen or occur within or on the septal wall. The adenomatous nodules appeared to develop through a combination of these processes. . . . [they were] composed of more or less closely packed columns of cuboidal or low columnar cells with relatively large nuclei. . . . few mitotic figures, . . . cytoplasm . . . occasionally contained small particles of phagocytosed material."

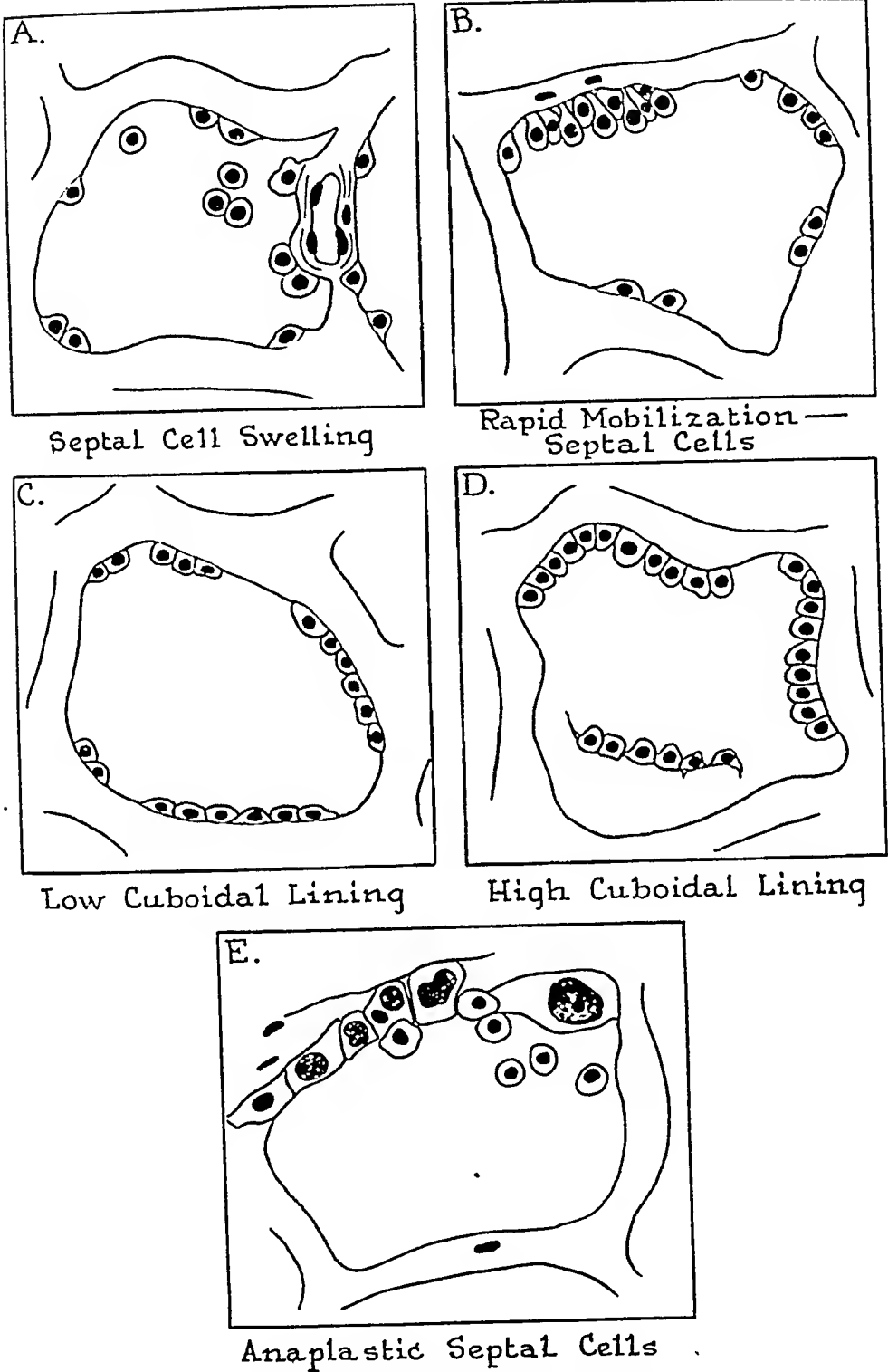
The authors hesitated to call these tumors "epithelial" because, after several transplants, they began to resemble spindle cell sarcoma. Wells, Slye and Holmes⁶² also observed sarcomatous tendencies in metastases of spontaneous lung tumors in mice. Andervont⁶⁶ and Breedis, Robertson, Osenkop and Furth⁶⁷ noted, in serial transplants, transformation of such tumors from an epithelial to a spindle cell sarcoma-like growth. The latter authors pointed out that the alveolar lining cells of mice "have potentialities of assuming both epithelial-like and sarcoma-like forms."

DISCUSSION

Developmental Stages of Lining. The alveolar cells accumulate readily under various pathologic conditions, as has been described in the preceding sections. All stages from simple swelling of the septal cells to the formation of a continuous epithelioid lining and to actual neoplastic growth (alveolar cell tumor) may be observed. We have illustrated the various stages diagrammatically in Text-Figure 1.

In early stages the nuclei of the septal cells become swollen and more prominent. They may be dark and homogeneous or pale, with a few chromatin granules and a dark nuclear membrane. The cytoplasm becomes more prominent, granular, or vacuolated and bulges into the alveolar lumen, although the cell remains continuous with the underlying ground substance (Text-Fig. 1, A). Such swelling can take place within a few hours, and has been verified in cases of massive acute postoperative atelectasis. Sometimes the septal cells appear to have enlarged and multiplied to a remarkable degree within a very short time. In such instances they may project into the lumen in clusters and their cytoplasm may become pear-shaped (Text-Fig. 1, B).

The next stage is actual lining. As the cells increase and their cytoplasm comes in contact with that of adjacent cells, they become low-cuboidal and form an indented, ribbonlike lining. At this point they assume an epithelioid appearance but usually remain attached to the



Text-Figure 1. Diagram of representative stages in septal cell activity.

underlying mesenchymal tissue. Special stains reveal no basement membrane in this or any other stage. The lining is apt to be incomplete, covering only a part of the alveolus (Text-Fig. 1, C).

Later the cells may become high-cuboidal with their attachment

to the underlying wall more delicate; the cells exfoliate occasionally in small numbers or in rows (Text-Fig. 1, D). One obtains the impression that to this stage the lining is not permanent and that it will disappear if recovery takes place.

Whenever the stimulating factor operates over a longer period of time, as in chronic inflammation, the proliferation is more apt to result in a complete, firmly attached lining, composed of higher cuboidal cells, which sometimes form papillary projections into the lumina. This lining appears more permanent, and such an assumption is supported by the fact that lined alveoli are found in old scarred areas.

In Text-Figure 1, E, anaplastic septal cells are shown with their attachment to the alveolar walls, as seen in an alveolar cell tumor (Fig. 11).

Although we have attempted to separate septal cell reactions into successive stages, we wish to emphasize that overlapping and combinations of these stages are commonly encountered.

Relation of the Mononuclear Phagocyte to the Alveolar Cell. There are several opinions as to the origin of the "dust cell" or alveolar phagocyte. According to one view, sponsored by Maximow and Bloom,⁸ Lang,¹⁶ Fried,¹⁹ Clements,⁶⁸ and others, the septal cells provide the majority of these phagocytes. Other opinions differ and assign the origin to either the cells of the blood stream or the capillary endothelium. Our material suggests that a high percentage of the mononuclear phagocytes arises from the septal cells. This study has not answered the question why the septal cells tend predominantly, under some conditions, to form alveolar linings; under others, to develop mainly into free phagocytes; and, in yet others, to manifest a combination of both features. It would appear that in certain acute inflammatory diseases or in the early stage of chronic inflammation, phagocytic properties are more pronounced. In these conditions, however, septal cell lining may be developed. The lining cells can become detached to form free phagocytes or they may remain attached to the wall and yet be phagocytic, as illustrated by occasional fat droplets and iron and dust particles in their cytoplasm. In the attached cells, these phagocytic properties are limited and are much less evident than in the free forms. Figure 16 is an example of swollen septal cells in a sheep; the cells are enlarged and are in the process of detachment from the wall to become free phagocytes. In old inactive conditions, particularly in pulmonary scars, the lining cells are inert and appear to have lost the power of phagocytosis and of producing phagocytes.

Relation of Septal Cell Proliferation to Specific Lung Regions. Lining formation is more readily found in certain locations than in others.

The septal cells in the alveoli subjacent to the visceral pleura show a pronounced tendency to proliferation, as mentioned earlier. Furthermore, the tendency is marked among the cells along the interlobular septa and in perivascular and peribronchial areas, but to a lesser degree. Macklin¹⁸ has demonstrated experimentally that the septal cells are more concentrated in the alveolar walls adjacent to these regions. This is of interest in its relation to the findings of Wells, Slye and Holmes,⁶² who observed in mice that spontaneous and experimentally produced lung tumors arose almost invariably in locations near the pleura.

Differentiation between Septal Cell and Bronchial Epithelial Lining. This distinction is important. We have observed both forms in man and in animals, and in most instances were able to differentiate between them without the aid of serial sections. Bronchiolar epithelial downgrowth into the alveolar ducts or alveoli may be of two types, squamous and columnar. The squamous type is easily distinguished histologically and is present in the form of irregular cell nests: this type of epithelium is metaplastic. In a few blocks which we studied serially, an early unsuspected squamous cell carcinoma was discovered. Columnar epithelial downgrowth is more important in its relation to our subject, because this type of epithelium may resemble the lining septal cell. We noted the differentiation best in the lungs of sheep that had suffered from chronic progressive pneumonia; this disease offered an opportunity to study both types of cells side by side in the same section. Bronchogenic epithelial downgrowth could be readily identified in most cases, because of the close relation to a central bronchus or bronchiole. The cells lining such spaces were high-columnar and were similar to bronchogenic epithelium, that is, the nuclei were oval or rod-like and not infrequently were arranged in layers; usually the cytoplasm was not clearly demarcated. Furthermore, the lumina of spaces lined by bronchogenic epithelium did not contain the mononuclear phagocytes to any such degree as that observed in zones of septal cell proliferation; the transition between the lining cells and mononuclear phagocytes was absent. Proliferating bronchiolar epithelium often covered spaces in a manner which produced thick collars of cells without evident cellular papillary projections. In summary, although the alveoli may become lined not only by autochthonous cells but also by bronchogenic epithelium, each type manifests fairly distinct characteristics.

Relation to Debatable Questions in Normal Histology. It is not our purpose to furnish conclusive evidence concerning debatable topics in normal pulmonary histology, since this study is based on pathologic material. Our findings, however, appear to support the view of Maxi-

mow and Bloom,⁸ Lang,¹⁶ Fried,¹⁹ Loosli,²⁰ and others, with respect to the structure of the normal alveolar wall, namely, that no true continuous epithelial lining exists in adult life. The capillaries appear to be contained in a ground substance in which occasional septal cells are present. The question whether these cells are of mesenchymal origin or are remnants of the fetal entodermal epithelium cannot be decided on the basis of our studies, although the first assumption appears to be preferable.

Histogenesis of the Alveolar Lining. Histologic findings indicate that the proliferative process leading to the production of lining takes place within the mesenchymal tissue of the alveolar wall. Mitotic figures, as found under experimental conditions (Macklin⁶⁹), are almost never observed. The lining cells become visible within the ground substance and proceed to cover the surface of the alveolar wall: thus a mesenchymal rather than an entodermal lining is formed. The genesis of cellular multiplication is not at all clear; proliferation of pre-existing septal cells certainly accounts for most of it, but participation of hematogenous cells cannot be excluded. At any rate, the potentialities of this mesenchymal tissue appear great, and large numbers of cells, presumably not present before, or at least not previously discernible, may become visible rapidly in the mesenchymal ground substance. The cellular mobilization is so striking that one is tempted to resort to Grawitz' ⁷⁰ theory of "slumbering cells" in the connective tissue.

Factors Stimulating the Formation of Lining. In small, early, bronchopneumonic foci one may observe variable, but more or less marked, transient septal cell swelling. In certain other forms of acute inflammation, notably those due to virus infections, the irritant factor is capable of producing septal cell proliferation and actual lining. The stimulus appears to be some change in circulatory conditions as a result of infection. Ricker ⁷¹ demonstrated that in prolonged disturbances of circulation characterized by marked slowing of the blood stream, called by him "peristatischer Zustand," stimulation of the mesenchymal tissue elements occurs. This may be the explanation, at least in part, for the septal cell stimulation and alveolar lining formation, particularly in chronic diseases. In alveolar cell tumors the causative force is obscure.

Interference with internal respiration appears to be an important factor in the formation of alveolar lining as mentioned by Aschoff.²³ He stated that alveoli that are collapsed or filled with exudate show marked tendency toward the proliferation of what he called "alveolar epithelium." The physicochemical aspects of this subject were considered by Young.⁷²

Functional Disturbances in Lined Alveoli. Lined alveoli are probably unable to function properly. Zeldes¹⁵ emphasized this on the basis of a study of the lungs of newborn infants, and believed that an extensive cuboidal lining was incompatible with respiratory function and led to the death of the infant soon after delivery. We have pointed out that in acute inflammatory conditions the lining probably disappears with recovery. The inert lining in the chronic inflammatory conditions described earlier may interfere with respiratory function. In scars the lined spaces are probably cut off from the bronchial tree and are nonfunctional.

SUMMARY

1. The pulmonary alveoli in man and in animals were studied with special regard to the character of the lining.
2. No evidence of continuous "alveolar epithelium" was observed in normal adult lungs; only occasional scattered septal cells were seen.
3. Epithelium-like lining cells were found under various spontaneous pathologic conditions.
4. The origin and development of the epithelium-like alveolar lining cells were discussed; their probable mesenchymal character was indicated.

We wish to express our appreciation to Drs. C. C. Macklin and H. G. Grady for many valuable suggestions.

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DESCRIPTION OF PLATES

PLATE III

- FIG. 1. Lobar pneumonia, stage of red hepatization, complicated by chronic passive congestion. Alveoli are lined by low cuboidal cells and filled with exudate. $\times 140$.
- FIG. 2. Chronic pneumonia. Alveolar walls are thickened and lined by cuboidal cells. $\times 280$.
- FIG. 3. Bronchopneumonia. Alveolar walls exhibit many swollen, prominent septal cells; lumina contain exudate with polymorphonuclear leukocytes, fibrin and occasional foamy phagocytes. $\times 175$.

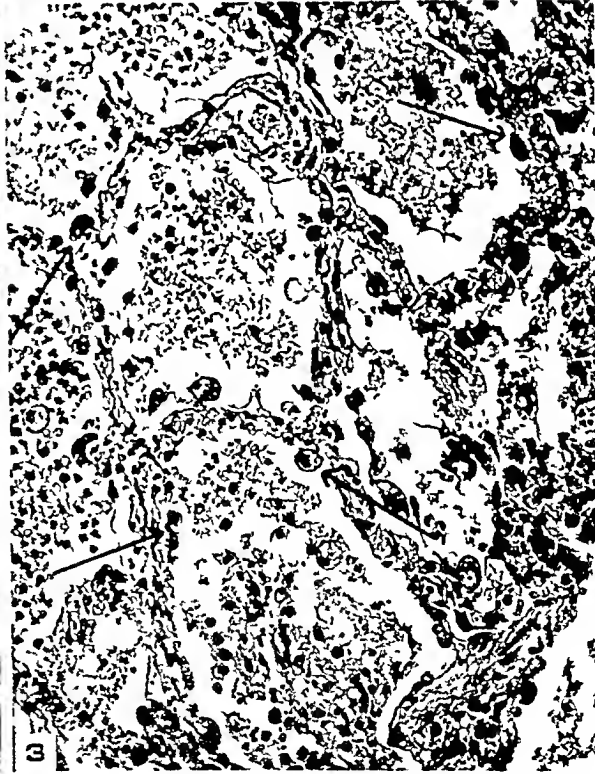
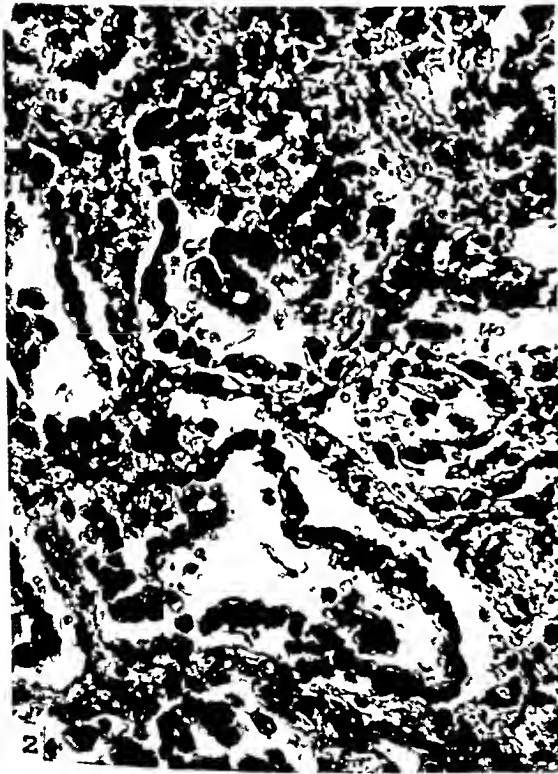
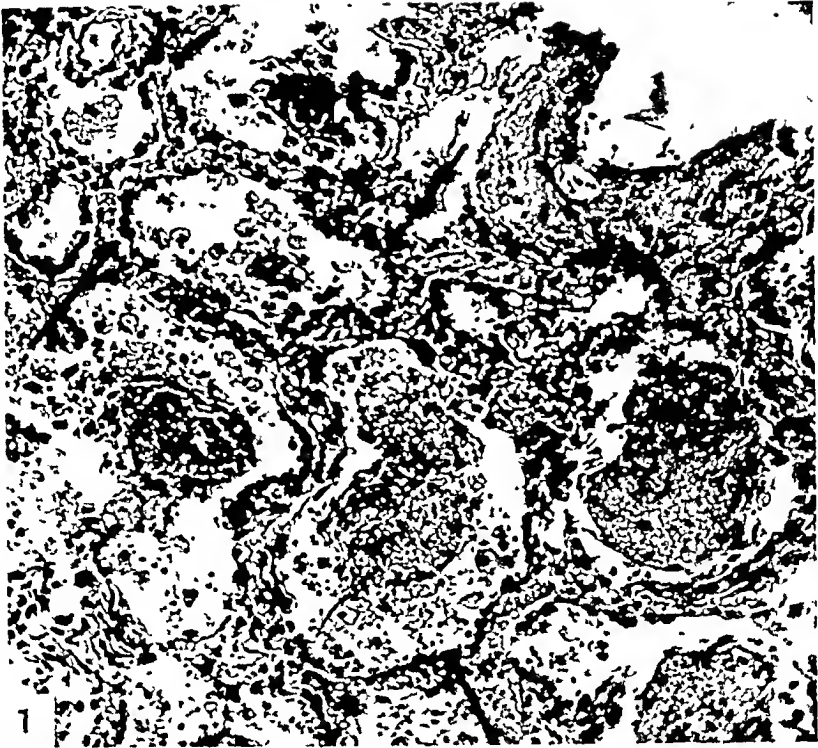


PLATE 112

- FIG. 4. Pneumonia complicating pertussis. Alveolar walls are lined by low, sometimes flattened, septal cells; many air sacs are atelectatic. Mononuclear cells predominate in the exudate. $\times 140$.
- FIG. 5. Chickenpox pneumonia. Alveolar walls show partly exfoliated lining. $\times 350$.
- FIG. 6. Lipoid pneumonia. Cuboidal lining is apparent in most alveoli. Lumina contain much free and phagocytosed fat. $\times 100$.
- FIG. 7. Pulmonary changes secondary to x-ray and radium treatment for malignant bronchial polyp. Alveolar walls are thickened and lined by low cuboidal cells. Foamy phagocytes are present in the lumina. $\times 155$.

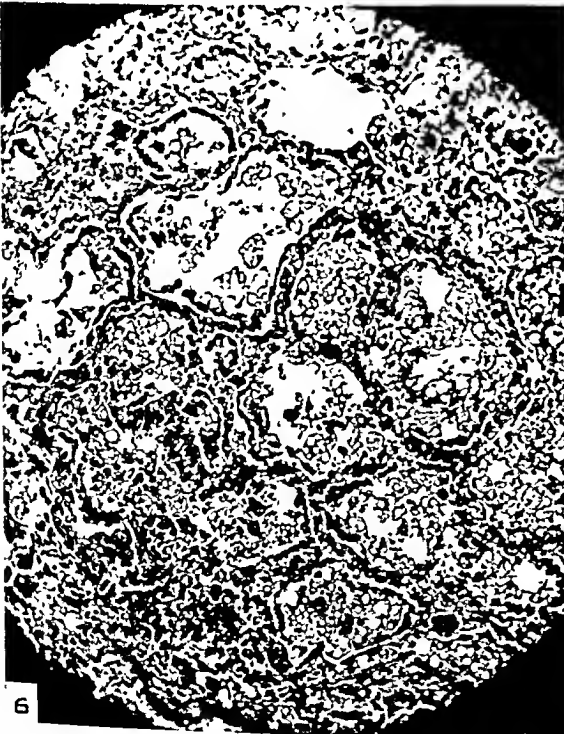
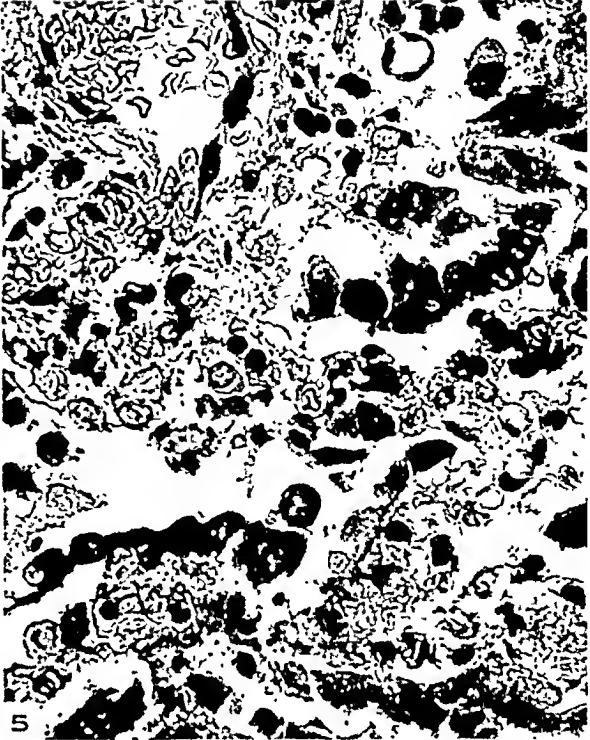


PLATE 113

- FIG. 8. Pulmonary tuberculosis. Marginal tuberculous granulation tissue is apparent around foci of necrosis. The adjacent alveoli are irregular and lined by continuous cuboidal cells. $\times 45$.
- FIG. 9. Pulmonary tuberculosis, area of acute exudation. Alveolar walls are congested, thickened and lined by continuous low and tall cuboidal septal cells. Phagocytes, round cells and occasional polymorphonuclear leukocytes are present in the exudate. $\times 160$.
- FIG. 10. Apical scar. Distorted or constricted alveoli are present in a fibrous background. Cuboidal lining is displayed in the majority of such alveoli. $\times 45$.
- FIG. 11. Alveolar cell tumor. Alveolus shows anaplastic lining with close relation to foamy phagocytes. $\times 360$.

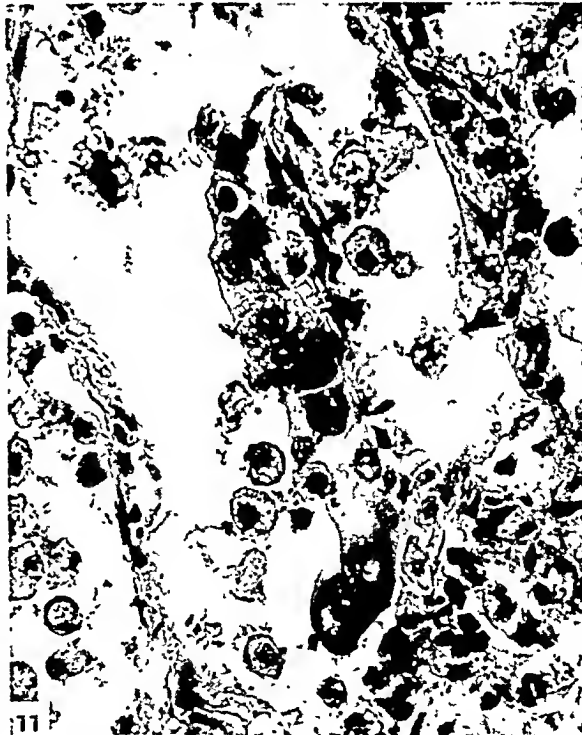
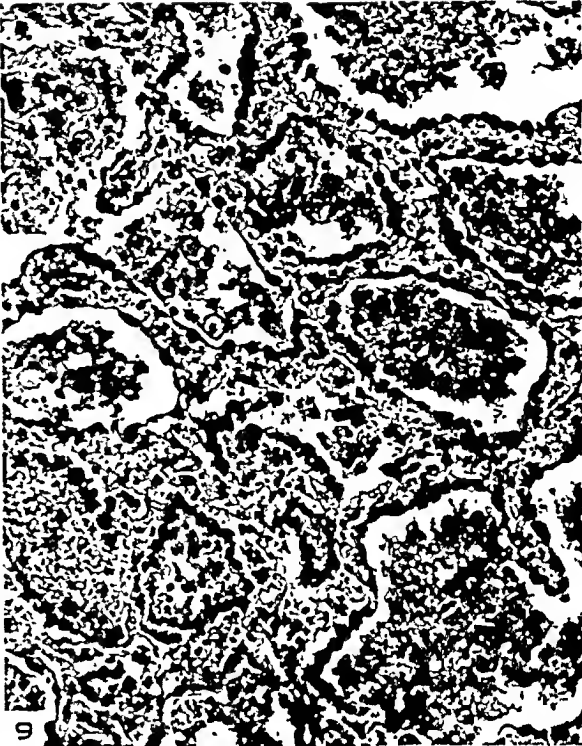
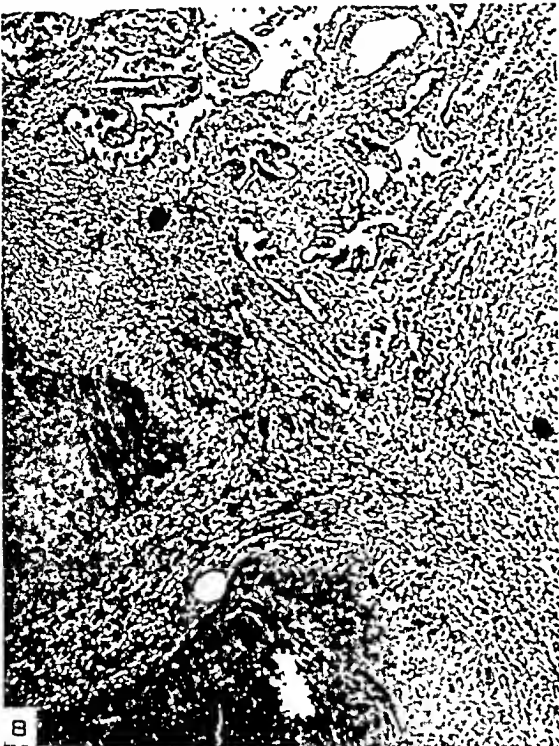
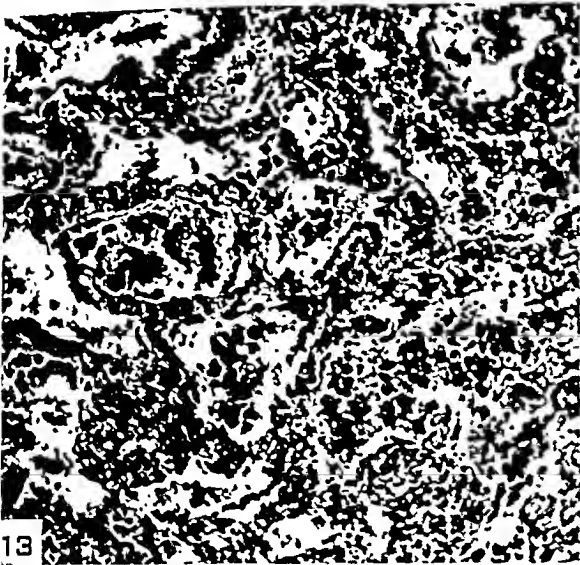
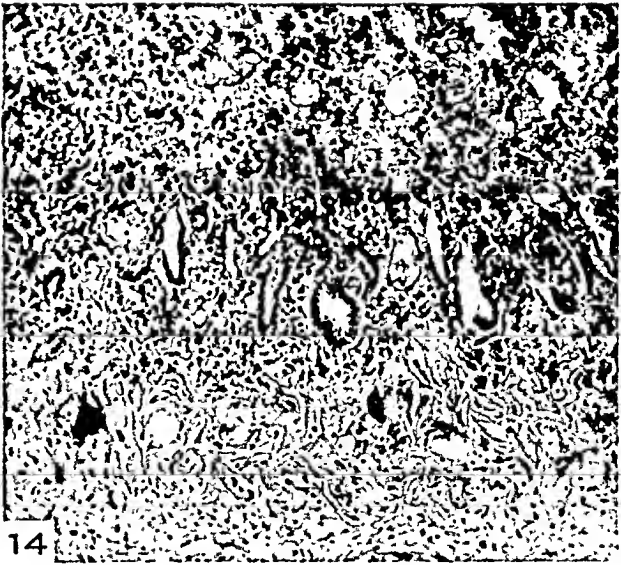


PLATE 114

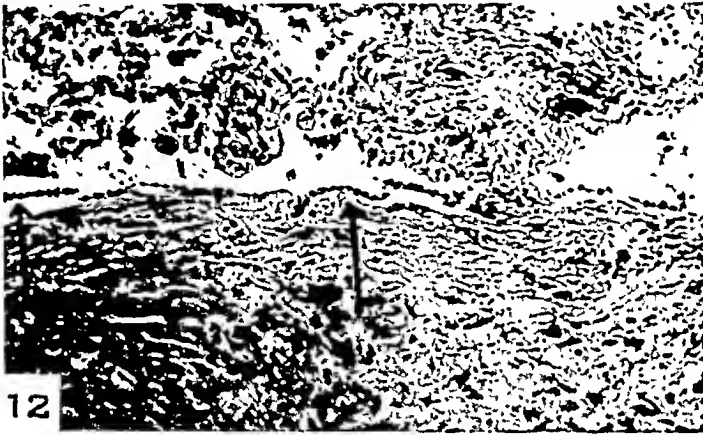
- FIG. 12. Silicotic nodule. Marginal alveoli are emphysematous and exhibit ribbon-like cuboidal lining on the surface adjoining the nodule. $\times 115$.
- FIG. 13. Chronic passive congestion. Alveolar walls are covered by cuboidal cells and the lumina filled with "heart lesion" phagocytes. $\times 95$.
- FIG. 14. Pleurisy. Visceral pleura is thickened and fibrous and subjacent alveoli are lined by cuboidal or columnar cells. $\times 110$.
- FIG. 15. Chronic progressive pneumonia in a sheep. Alveoli and alveolar ducts are lined by cuboidal or low columnar cells forming papillary projections into the lumina. Alveolar walls are thickened and show round cell infiltration. $\times 175$.
- FIG. 16. Acute pneumonia in a sheep. Alveoli have pear-shaped septal cells, projecting into the lumen in clusters. Polymorphonuclear leukocytes and foamy phagocytes are also present. $\times 410$.



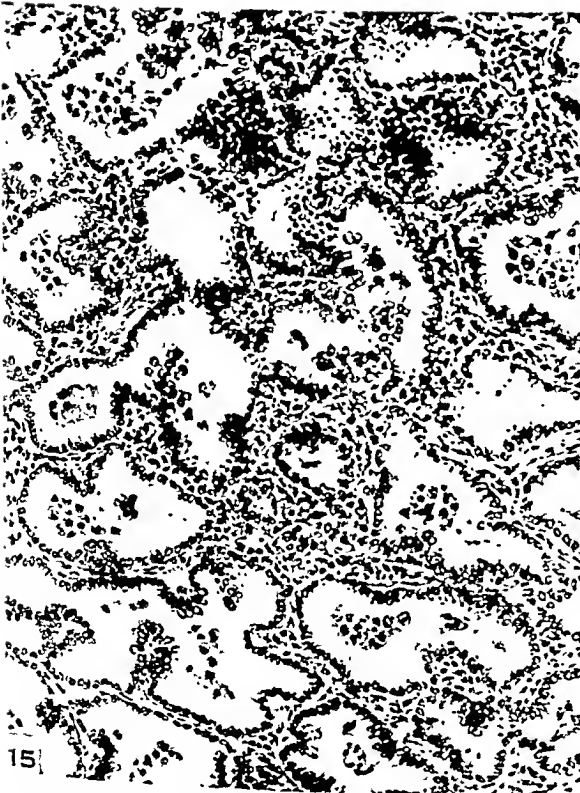
13



14



12



15



16

EXPERIMENTAL BRAIN TUMORS

II. TUMORS PRODUCED WITH BENZPYRENE*

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In experiments with pure crystalline 20-methylcholanthrene implanted in pellet form in the brains of C_3H male mice, a variety of gliomas and intracranial sarcomas were produced which have already been reported.¹ More recently another hydrocarbon, benzpyrene, was utilized for intracerebral implantation in both male and female mice of the C_3H strain, and with this chemical carcinogen also intracranial neoplasms were produced. These experiments were previously reported in preliminary form² and now are the basis of this detailed communication.

Other investigators have employed benzpyrene in attempts to produce brain tumors with invariably negative results in regard to gliomas. In 1936, Oberling, Guérin and Guérin³ applied this hydrocarbon in crystalline form to the brains of 10 adult rats, of which 3 animals developed pituitary adenomas after 10 months of survival. Another rat which received 1 drop of a 1:1000 oily solution of benzpyrene intracerebrally developed an atypical epitheliomatous tumor in the anterior lobe of the hypophysis. The authors maintained at that time that the tumors were produced by the chemical carcinogen because of the rarity of spontaneous pituitary tumors in rats. Three years later, however, Oberling, Sannié, Guérin and Guérin⁴ conducted more extensive experiments with an oily solution of benzpyrene in guinea-pigs, rabbits, hens and rats in an effort to duplicate these results. They also implanted crystals of this chemical in the brains of 40 rats. The negative results forced them to confess that pituitary adenomas could not be induced by these methods and that the previously reported adenomas were spontaneous. Although the experiments were continued for over a year, no gliomas and only 12 intracranial and extracranial sarcomas developed.

In 1937, Askanazy⁵ reported finding a piece of cartilage in the ventricle of a rabbit injected intracerebrally with crushed rabbit fetal tissue and with benzpyrene in olive oil. This animal lived only 22 days but another, treated similarly, which survived 8 weeks, was said to have had a chondroma in the lateral ventricle. One rabbit received an intracerebral injection of benzpyrene in beef fat and died 8 months

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and 5 days later with a "chondrosarcoma" in the cerebellum. Considerable doubt may be expressed as to the neoplastic nature of any of these cartilaginous masses, for at least in the first two instances the cartilage probably represented part of the crushed fetal tissue.

Bertrand and Gruner⁶ suspended benzpyrene in lanolin, paraffin oil and vaseline, injecting this material in doses of 5 to 15 mg. intracerebrally in rabbits. Although in certain instances a giant nuclear glial response appeared at the sites of injection, true gliogenous neoplasia was not produced. In a personal communication to Seligman and Shear,⁷ Zylberszac stated that he failed to obtain tumors in 35 rats in which he introduced crystalline benzpyrene subdurally. In a similar communication,⁷ Scherer revealed that he injected 0.1 cc. of a 1 per cent lard solution of benzpyrene into the frontal lobes of 70 white rats and gave 50 of the animals more than one injection. Granulomas were found in all cases but no neoplasms were seen, and lard itself caused the same reaction.

In view of the success that attended the experimental production of brain tumors with pellets of pure methylcholanthrene in C_3H mice,¹ the essentially negative results of all of the experiments with benzpyrene were surprising. But it must be noted that no one had used the mouse as the experimental animal and, further, that usually the carcinogen was diluted or contaminated with oily vehicles that set up foreign body reactions. Even when crystals of benzpyrene were employed they were not compressed into pellets for intracerebral implantation. In the following experiments, which took account of these factors, namely, the animal species and the pellet form of the carcinogen, brain tumors of a large variety were produced.

EXPERIMENTAL METHOD

A total of 51 mice of the C_3H strain, representing both sexes in about equal numbers, was employed in these experiments. The animals were between 3 and 4 months of age. Their care and diet were in every way similar to those of the earlier experiments with methylcholanthrene.¹ Likewise, the anesthesia, operative procedure and after-care were the same, with the exception that the chemical carcinogen was implanted in the right cerebral hemisphere in each instance. As in the earlier experiments, the tumors of doubtful diagnosis which developed were transplanted to the subcutaneous tissues of other mice of the same strain. The help that was derived from these transplants in classifying the tumors has been utilized in the presentation of the data which follow.

The necropsy and histologic technics were similar in these and in

the experiments with methylcholanthrene. The carcinogen was 3,4-benzpyrene, obtained from Meurice, Union chimique belge, Brussels, Belgium. Without further purification, the canary yellow crystals were heated gently in a small beaker until they fused. When cooled, cubes of about 1 mm. diameter were cut with a knife and were employed, without further treatment, for implantation in the mouse brains.

RESULTS

Of the 51 mice at the start of the experiments, 47 survived into the tumor-bearing age. The tumor incidence and classification are presented in Table I.

TABLE I
Tumor Incidence and Classification

Total number of animals	47
Negative for tumor	19
Total number of tumors	28
Gliomas	14
Astrocytoma	1
Ependymoblastoma	4
Glioblastoma multiforme	3
Medulloblastoma	1
Oligodendroglioma	1
Unclassified glioma	4
Sarcomas	13
Extracranial fibrosarcoma	2
Intracranial fibrosarcoma	9
Rhabdomyosarcoma	2
Mixed glioma and sarcoma	1

These experiments were completed after 458 days upon the death of the last surviving mouse. The average survival time for the animals which developed gliomas was 276 days as compared to 245 days for those which developed sarcomas. The related data are presented in Table II.

Many of the tumors produced with benzpyrene were similar to those which resulted from the intracranial implantation of pellets of methylcholanthrene. Since the latter were described and illustrated extensively in the earlier paper,¹ the same types of neoplasms produced with benzpyrene will be dealt with only briefly at this time. More attention will be given those tumors, like the ependymoblastomas, which have not been described previously.

Gliomas

Astrocytoma. Mouse 21. A gray, opaque, poorly defined tumor was present at the site of the benzpyrene pellet in the right cerebral hemisphere. The cells formed no definite pattern, being arranged in helter-skelter fashion. Each consisted of a small, deeply stained round

nucleus and inconspicuous cytoplasm with multipolar processes. There were few cells in mitotic division. Minute portions (1 mm. in diameter) of this tumor were transplanted into the subcutaneous tissues of the right flanks of 4 C₃H mice, but only one transplant grew after 3 months. The remaining three failed to grow even after 4 more months. Subtransplants were made of the one positive transplant, yielding two

TABLE II
Survival Time of Animals with Various Tumors

<i>Gliomas, 14 animals: average survival time, 276 days</i>		
Mouse no.	Days' survival	Tumor type
10	359	Unclassified
12	286	Unclassified
17	222	Glioblastoma multiforme
19	384	Ependymoblastoma
21	238	Astrocytoma
22	230	Oligodendroglioma
26	329	Glioblastoma multiforme
35	189	Glioblastoma multiforme
37	272	Unclassified
38	230	Ependymoblastoma
40	319	Ependymoblastoma
43	174	Ependymoblastoma
50	452	Unclassified
52	183	Medulloblastoma
<i>Sarcomas, 13 animals: average survival time, 245 days</i>		
Mouse no.	Days' survival	Tumor type
3	208	Intracranial fibrosarcoma
5	267	Intracranial fibrosarcoma
6	222	Rhabdomyosarcoma
8	153	Intracranial fibrosarcoma
13	239	Rhabdomyosarcoma
14	153	Extracranial fibrosarcoma
16	458	Intracranial fibrosarcoma
18	458	Intracranial fibrosarcoma
20	171	Intracranial fibrosarcoma
30	170	Intracranial fibrosarcoma
45	275	Extracranial fibrosarcoma
47	147	Intracranial fibrosarcoma
51	267	Intracranial fibrosarcoma
<i>Mixed glioma and sarcoma</i>		
Mouse no.	Days' survival	Tumor type
1	208	Mixed glioma and sarcoma

positive "takes" out of 4 animals. The cells in the tumor transplants were polygonal and had many polar processes. They were often arranged around irregular spaces which were filled with pale pink (in hematoxylin and eosin preparations), colloid-like material.

Ependymoblastoma. Mouse 19. At necropsy the brain of this animal had a small tumor nodule in the right lateral ventricle attached to the lateral wall and infiltrating the right cerebral hemisphere. In places the ventricular ependyma was replaced by broad bands of tumor

cells which were carrot-shaped and invaded the choroid plexus. The cells which infiltrated the hemisphere were frequently arranged as collars around blood vessels and formed rosettes whose centers consisted of granular debris. Numerous cells were in mitotic division.

Mouse 38. Two days before this animal died it became inactive, appeared to be sick and developed a deformity of the head. At necropsy the cranial sutures were found separated and the occipital bone thin and bulging. A roughly spherical tumor mass was found in the right lateral ventricle with the pellet of benzpyrene (B.P.) still present in the upper part of the tumor (Fig. 1, B.P. 38). Microscopically the ventricular ependyma was found to have proliferated in several places. The cells were typically carrot-shaped and were often arranged around blood vessels.

Subcutaneous transplants of this neoplasm in 4 C₃H mice yielded three positive "takes" in less than a month. Subtransplantation was carried out twice with all of the animals showing tumor growth in less than 30 days. The tumor transplants were even more characteristic of ependymoblastoma than the original neoplasm. All of the cells were carrot-shaped and formed typical rosettes. Many cells were in mitotic division.

Mouse 40. The head of this animal became asymmetrical 2 days before it died, when the cranial sutures were found widely separated and the right half of the cranium was soft. The tumor was situated in the right cerebral hemisphere, of which the ventricle was dilated and filled with an excess of clear fluid. The choroid plexus as well as the leptomeninges were infiltrated with tumor cells. Pear-shaped cells radiated from blood vessels and formed rosettes with centers that were fibrillary, acellular and pink-staining. Even in the meninges the tumor cells formed typical rosettes (Fig. 2). Mitoses were present in abundance. Subcutaneous transplants of this neoplasm gave rise to growths which were typical of ependymoblastoma (Fig. 3).

Mouse 43. Four days before this animal died it began to run in circles and to fall to the right. Its head was deformed due to separation of the cranial sutures. A tumor replaced the posterior half of the right cerebral hemisphere and resulted in a generalized dilatation of the ventricular system. The tumor cells were carrot-shaped and were arranged in rosette formations with many cells in mitotic division. This tumor was kept alive through 4 generations of transplants involving a total of 16 animals, when further transplantation was arbitrarily stopped. Every transplant grew, reaching a minimal diameter of 1 cm. in approximately 1 month's time. The characteristic cellular pattern was maintained through all generations of transplants.

Glioblastoma Multiforme. Mouse 17. A small palpable nodule became evident beneath the scalp in the right parietal region of this animal 10 days before death. This tumor grew rapidly and at necropsy was found to arise from the right cerebral hemisphere, projecting beneath the scalp through an erosion of parietal bone (Fig. 1, B.P. 17). Microscopically the tumor was diffusely infiltrating glioma with evidence of invasion of the meninges as well as of the ventricular system. The cells were pleomorphic and there were many multinucleated giant cells as well as cells in mitotic division. A characteristic arrangement of the cells was in pseudopalisades around zones of necrosis.

Three generations of subcutaneous transplants of this tumor were kept alive, the first transplants showing evidence of growth in about 6 weeks and the latter two, in a little over 3 weeks. The transplanted tumors failed to develop giant cells and pseudopalisades. The structural pattern assumed by the cells resembled that of a piloid astrocytoma.

Mouse 26. This animal died without revealing any external evidence of an intracranial neoplasm. Such a tumor, however, was found at necropsy surrounding the pellet of benzpyrene in the subcortex of the right cerebral hemisphere. Microscopically this neoplasm was a typical glioblastoma multiforme, with multinucleated giant cells, spongioblasts and necrotic foci surrounded by cells of pseudopalisade formation. Small round cells formed collars around blood vessels in the tumor.

Mouse 35. At death there was no deformity of the head of this animal. The skull was intact, but there was a slight separation of the cranial sutures. A gray, opaque tumor containing minute hemorrhages was present in the right parieto-occipital region of the brain (Fig. 1, B.P. 35). Neoplastic cells were found invading the choroid plexus microscopically. There was a great diversity of cell form and size, with many elements in mitotic division. Occasional multinucleated giant cells were present. Spindle-shaped spongioblasts formed pseudopalisades around foci of necrosis.

Medulloblastoma. Mouse 52. There was nothing on external examination of the skull of this animal to indicate the tumor which was, situated in the parieto-occipital region (Fig. 1, B.P. 52). When sectioned, the central portion of the neoplasm was found to be hemorrhagic. There was no neoplastic involvement of the cerebellum. The cells had a vague resemblance to lymphocytes: the nuclei were round and deeply stained in inconspicuous cytoplasmic bodies. Mitotic figures were exceedingly numerous. The cellular infiltration receded so

gradually that there was no real demarcation between the neoplasm and the adjacent nervous tissue. The perivascular spaces of many cerebral vessels contained nests of tumor cells. In the main body of the new growth as well as in its perivascular extensions, a tendency to form pseudorosettes was a conspicuous feature of the cells. In spite of the rather unorthodox location of this neoplasm, its cellular composition and structural pattern permit no diagnosis other than medulloblastoma.

Oligodendroglioma. Mouse 22. Nearly 2 weeks before this animal died, a small mass was visible on the right side of its head. This increased in size only slowly, and at necropsy was found to emanate from the burr hole in the skull. The benzpyrene pellet was found lying superficially in the cortex of the right occipital lobe and did not appear to be surrounded by tumor. On serial microscopic examination, however, the region of the pellet was occupied by typical oligodendroglia, with centrally situated round nuclei and colorless cytoplasm which produced the appearance of a perinuclear halo in each cell.

Within 2 months after subcutaneous transplantation of this tumor into 4 C₃H mice, all animals showed evidence of a positive "take." Subtransplants of the tumor from the flank of 1 animal into 4 others yielded three positives at the end of 2 months. Further subtransplantation yielded once more three out of four positive "takes" in a little less than 2 months. With minor variations the microscopic structure of all of the transplants resembled the original brain tumor. The variant which appeared in 2 animals consisted of bands of spindle-shaped mesodermal cells and reticulin in addition to the oligodendroglia.

Unclassified Glioma. Mouse 10. This animal died without displaying a change either in its behavior or the shape of its head. Necropsy, however, revealed a gray-brown tumor in the right cerebral hemisphere. The cells were nearly all spindle-shaped and had elongated nuclei. Frequently they were arranged in concentric rings or bands around blood vessels, resembling spongioblasts quite closely. Mitotic figures were moderately numerous. There was invasion of the choroid plexus. Preparations stained by the Wilder method failed to disclose connective tissue reticulin fibers. The unexpected death of this animal precluded further study of this neoplasm by the transplantation method.

Mouse 12. The unexpected death of this animal occurred without any cranial deformity or change in behavior. The intact skull having been removed, a gray-brown tumor was found in the posterior half of the right cerebral hemisphere. When sectioned, the central portion of the tumor was seen to be hemorrhagic. The histologic appearance of the

neoplasm varied in different parts. In some zones the cells were polygonal and had abundant pink cytoplasm and large, chromatin-rich nuclei. In other zones bizarre-shaped, multinucleated giant cells were conspicuous. In still others, mononuclear cells with foamy cytoplasm and cytoplasmic vacuoles predominated. These various cellular elements infiltrated the nervous tissue diffusely. Perivascular lymphocytic infiltration was noted in several places. Cells in mitotic division were seen rather frequently. In spite of certain resemblances to glioblastoma multiforme, this glioma is left unclassified. Transplants were not available to aid in diagnosis.

Mouse 37. This animal became inactive and appeared to be sick 1 week before it died. It held its head to the left, but there was no cranial deformity. Section of the brain disclosed a dilatation of the ventricular system, slightly more marked in the right lateral ventricle (Fig. 4). A gray, translucent tumor nodule was present at the site of the benzpyrene pellet which lay embedded in the septum pellucidum just beneath the corpus callosum. Serial microscopic preparations of the brain showed that the tumor nodule was composed mainly of large, round mononuclear glial cells (Fig. 5). Some cells were considerably smaller; a few had short, thick cytoplasmic processes; some were in mitotic division. Here and there a cell was multinucleated and of giant size. Scattered throughout the brain were vessels with small round cells in their perivascular spaces. Tumor cells had infiltrated the leptomeninges and lay in a cluster within the third ventricle (Fig. 7). Preparations of a portion of the tumor nodule stained by the Wilder method for mesodermal reticulin were entirely negative (Fig. 6).

Although certain features of this neoplasm were suggestive of glioblastoma multiforme, its general microscopic appearance did not warrant this classification. Moreover, the transplantation experiments gave little aid in this direction. In the first transplant generation, 3 out of 4 mice developed subcutaneous tumors, but only after 3 months. All 4 animals of the second transplant generation developed tumors within 5 weeks, but in no instance were the transplants more characteristic microscopically than the original brain tumor.

Mouse 50. There was no microscopic evidence of a neoplasm in the brain at necropsy. Microscopic serial sections of the entire cerebrum revealed a gliogenous tumor adjacent to the pellet space in the subcortex of the right hemisphere. A moderate number of glial cells were in mitotic division and there was perivascular tumor infiltration in nearby regions. This new growth could not be identified further and because it was not recognized in the gross, no transplants were made.

Sarcomas

Extracranial Fibrosarcoma. Mice 14 and 45. In both instances the malignant neoplasms, showing an abundance of reticulin fibers, were entirely extracranial and, therefore, not within the scope of this report. Each seemed to arise from the subcutaneous tissues of the scalp at the sites of the craniotomy wounds and probably arose as the result of the extrusion of the pellet into the burr hole.

Intracranial Fibrosarcoma. Mice 3, 5, 8, 16, 18, 20, 30, 47 and 51. With the exception of mouse 18, which represents a special case, all of the animals of this group developed tumors beneath the intact scalp at the sites of craniotomies. The new growths grew rapidly and caused characteristic deformities of the head (Fig. 8). They all arose intracranially, apparently from the meninges, compressing the adjacent brain tissue. In each instance they mushroomed through the craniotomy opening to their extracranial position beneath the scalp, the intracranial and extracranial portions of the tumor often being connected by a narrow pedicle which was the part that passed through the burr hole (Fig. 9, B.P. 8, B.P. 20 and B.P. 30). In several instances the tumors arose from the meninges in the longitudinal fissure, in others from the cerebral coverings over the right hemisphere. They were always in contact with the benzpyrene pellet, rather well circumscribed and sharply demarcated from the adjacent nervous tissue which they failed to invade.

Microscopically they were composed of mesodermal cells of spindle shape, usually arranged in parallel rows. Nuclear division by mitosis was often present, as were also multinucleated cellular elements. Each tumor had an abundant reticular stroma which was easily impregnated with silver by the Wilder method. In transplants these neoplasms grew rapidly and maintained their microscopic characteristics.

Mouse 18 did not develop a cranial deformity. At necropsy the benzpyrene pellet was found embedded in the subcortex of the right cerebral hemisphere, but no evidence of tumor was visible to the naked eye. Microscopically, however, spindle-shaped cells, many in mitotic division, were present near the pellet and invaded the overlying pia-arachnoid. Although sharply demarcated from the cerebral tissue, these cells invaded the sheaths of parenchymal vessels and also infiltrated the choroid plexus. They produced a fibrillary reticulum which was argentophilic in Wilder preparations. In contrast, therefore, to the other intracranial sarcomas this neoplasm had the earmarks of a primary cerebral sarcoma originating from the perivascular sheaths.

Rhabdomyosarcoma. Mice 6 and 13. Both of these tumors were

entirely extracranial and were recognized as swellings in the temporal region during the lives of the animals. They arose apparently from the temporal muscles and consisted of elongated cells with oval nuclei, large bodies of deeply eosinophilic cytoplasm and interlacing fibrous processes. Scattered throughout the tumor were groups of large oval or round cells with two or more huge vesicular nuclei. Although these cells suggested strongly a striated muscle origin, striations were not demonstrable in their cytoplasm.

Mixed Glioma and Sarcoma

Mouse 1. A tumor mass appeared in the subcutaneous tissues of the scalp in the right parietal region 4 days before this animal died. At necropsy, the extracranial portion of the neoplasm was firm and gray and resembled the extracranial sarcomas. But that part of the neoplasm which was intracerebral was softer in consistency and poorly differentiated from the neighboring brain tissue (Fig. 9, B.P 1). That there were two different tumors became apparent from the microscopic study. The extracerebral tumor consisted of spindle-shaped cells with pale, oval nuclei, each having a single nucleolus. These cells lay in sheets and in whorls and produced a dense reticular stroma which was argentophilic. This was obviously a sarcoma.

The infiltrating intracerebral tumor was of gliogenous origin and consisted of small round cells, with dark homogeneous nuclei, and no reticulin fibers. The cells were scattered helter-skelter in no characteristic fashion. Small foci of necrosis were present but failed to produce any alignment of tumor cells around them. Mitotic figures were present in moderate numbers.

Unfortunately, only the extracranial neoplasm was employed for transplantation. These transplants grew exceedingly rapidly, all of them reaching a minimum size of 1 cm. in approximately 2 weeks. A total of 16 mice were used in four series of 4 animals each, and all of their tumors were sarcomas. Failure to employ the intracerebral tumor for transplantation precluded the opportunity for a more definite diagnosis and hence it must be listed as an unclassified glioma.

COMMENT

Approximately 50 per cent (24 out of 47) of the mice which had benzpyrene implanted in their brains developed intracranial neoplasms, excluding the two instances of extracranial fibrosarcoma and the two of rhabdomyosarcoma. This incidence compares favorably with that of tumors induced by methylcholanthrene, which was 46 per cent of C₃H mice.¹ Of the 24 benzpyrene-induced intracranial neo-

plasms, half were gliomas. This is in contrast to the apparent ability of pure methylcholanthrene to induce about five times as many gliomas as intracranial sarcomas. The sex of the animals appears to be of no importance in influencing the tumor incidence with benzpyrene; tumor-bearing mice were about equally divided between the sexes.

An effort was made in these experiments to implant the carcinogen rather deeply in the brain tissue, and in four instances where this resulted in the deposition of the pellets within the ventricular system, ependymoblastomas developed. The concept is thereby strengthened that the site of origin of the brain tumor influences its type.

Attention is called to the fact that a medulloblastoma developed in the right cerebral hemisphere, a very unusual site for this type of tumor. This experience, however, was paralleled in the experiments with methylcholanthrene, in which one medulloblastoma also was found in the cerebrum.

There was but little difference in the time required for the development of the gliomas and sarcomas. The average survival time of the animals with tumors of the first variety was 276 days, and of those with sarcomas, 245 days. This difference of a month may be even less significant since it represents the survival time of the animals rather than the appearance time of the neoplasms. In general, gliomas grow somewhat more slowly than sarcomas and, therefore, the survival of animals with tumors of the former type would naturally be longer.

The growth behavior of the gliomas was distinct from that of the sarcomas, as a comparison of Figures 1 and 9 will disclose. Whereas the gliomas infiltrated and faded imperceptibly into the brain tissue, the sarcomas were distinctly circumscribed and sharply demarcated from the nervous parenchyma.

SUMMARY

Pellets of 3, 4-benzpyrene were implanted in the right cerebral hemispheres of 47 C_3H mice of both sexes.

Twenty-eight tumors developed, of which 14 were gliomas; 9 were intracranial fibrosarcomas; 2 were extracranial fibrosarcomas; 2 were extracranial rhabdomyosarcomas; 1 was a mixed glioma and sarcoma. The types of glioma were: astrocytoma, ependymoblastoma, glioblastoma multiforme, medulloblastoma and oligodendroglioma. Four gliomas were unclassified.

The appearance of ependymoblastomas around pellets implanted in the ventricular system lends support to the concept that the *type* of glioma is a function of the *site* of its development.

A significant difference in the survival time of mice with gliomas

and intracranial sarcomas induced with benzpyrene was not found. This is all the more true of the rates of development of these two types of neoplasms.

The macroscopic appearance of the gliomas distinguished them from the intracranial sarcomas. The former were infiltrative and poorly demarcated from the brain tissue; the latter were distinctly circumscribed.

Subcutaneous transplantation of the intracranial tumors was found to be a valuable aid in the study and classification of these new growths.

The present experiments represent the first successful attempts to induce gliomas with benzpyrene.

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DESCRIPTION OF PLATES

PLATE 115

FIG. 1. *Mouse 17*. Glioblastoma multiforme. Drawing of hemorrhagic, infiltrating tumor in right cerebral hemisphere. *Mouse 35*. Glioblastoma multiforme. Drawing of gray, opaque and partly hemorrhagic, infiltrating tumor in right occipital lobe. *Mouse 38*. Ependymoblastoma. Drawing of tumor surrounding pellet of benzpyrene (B.P.) in ventricle. *Mouse 52*. Medulloblastoma. Drawing of hemorrhagic tumor mass infiltrating the posterior half of the right cerebral hemisphere.

FIG. 2. *Mouse 40*. Ependymoblastoma. Photomicrograph of tumor in cerebral leptomeninges. Typical rosettes and numerous mitoses are seen. Hematoxylin and eosin stain. $\times 375$.

FIG. 3. *Mouse 40*. Ependymoblastoma. Photomicrograph of subcutaneous tumor transplant. Hematoxylin and eosin stain. $\times 375$.



B.P. 3



B.P. 17



B.P. 38

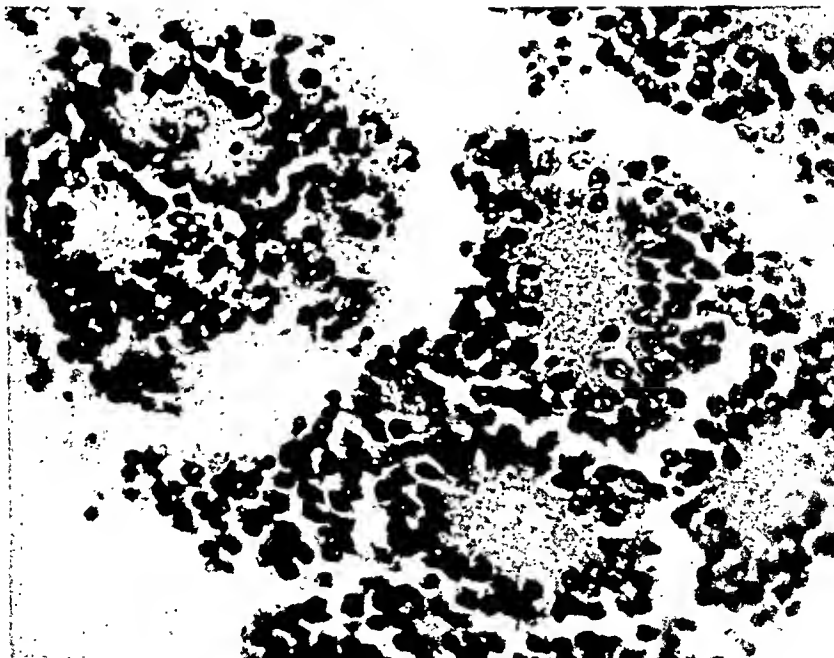


B.P. 52

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2



3

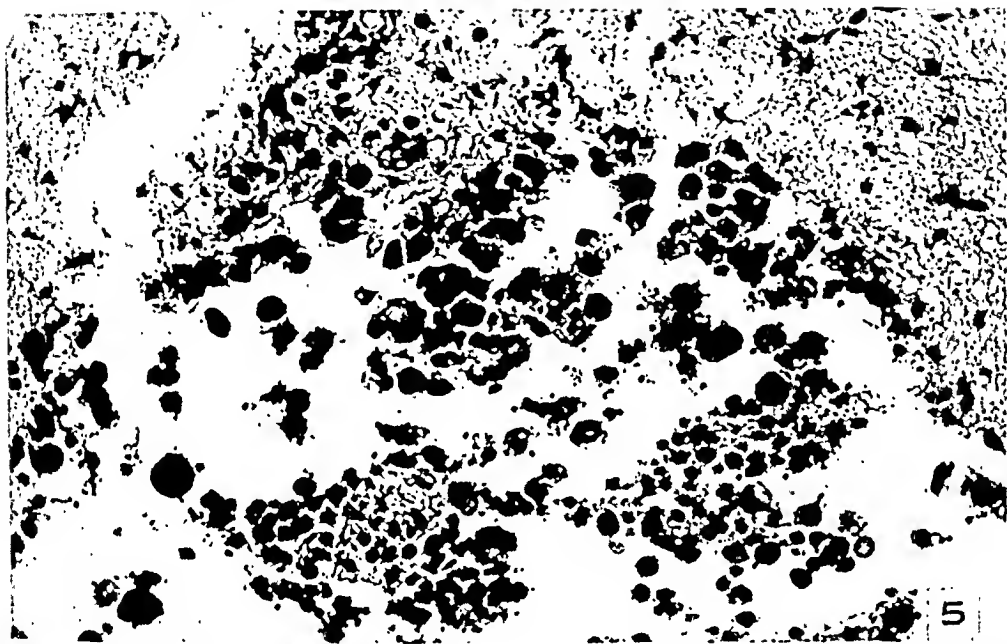
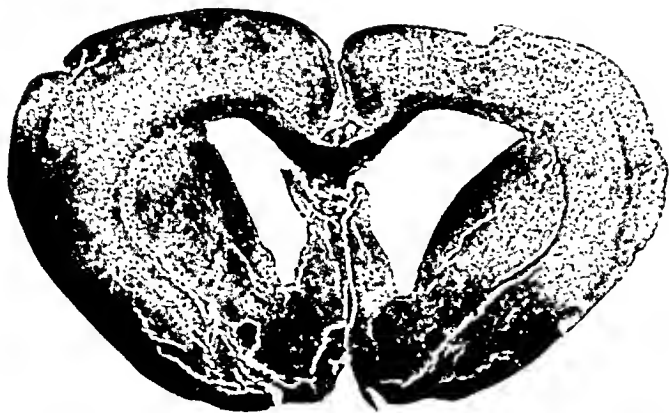
Zimmerman and Arnold

Brain Tumors Produced with Benzpyrene

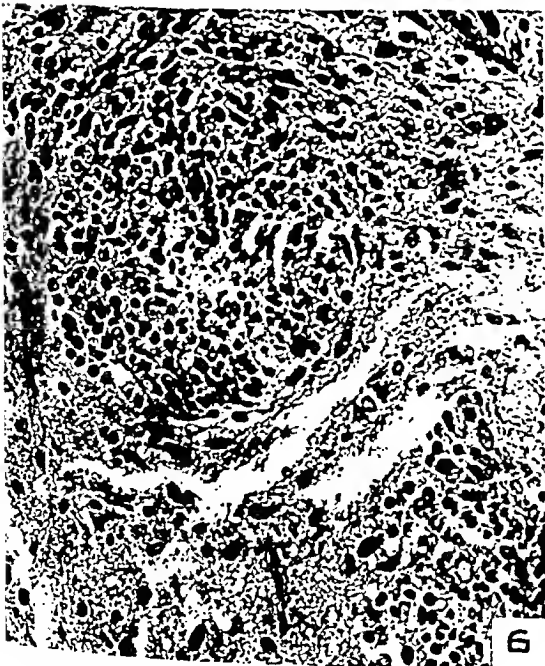
PLATE 116

- FIG. 4. Mouse 37. Unclassified glioma. Transverse section of brain showing ventricular dilatation and location of tumor in septum pellucidum. Hematoxylin and eosin stain. $\times 6$.
- FIG. 5. Mouse 37. Unclassified glioma. Tumor cells in corpus callosum. Hematoxylin and eosin stain. $\times 200$.
- FIG. 6. Mouse 37. Unclassified glioma. Tumor nodule stained by the Wilder method, showing absence of reticulin fibers. $\times 200$.
- FIG. 7. Mouse 37. Unclassified glioma. Tumor nodule in third ventricle. Hematoxylin and eosin stain. $\times 100$.

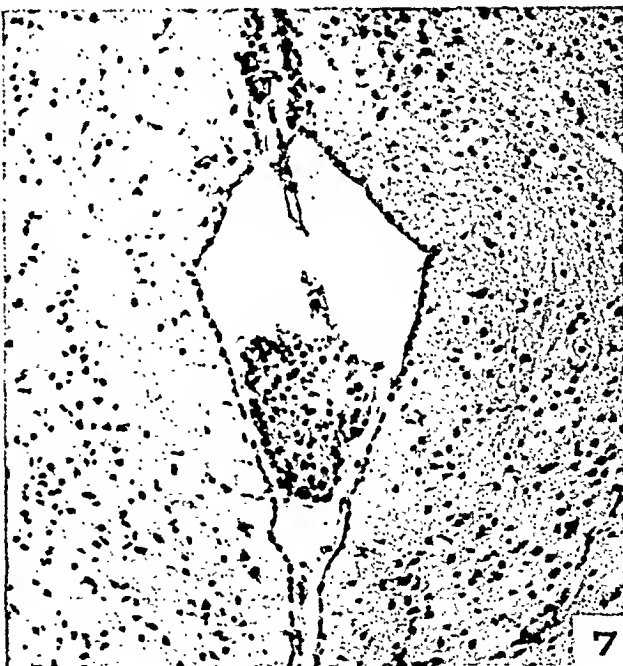
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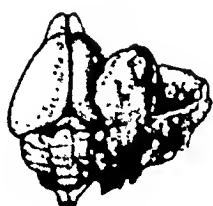
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PLATE 117

FIG. 8. Mouse 47. Characteristic deformity of head due to subcutaneous extension of intracranial fibrosarcoma through burr hole.

FIG. 9. *Mouse 1*. Mixed glioma and sarcoma. The infiltrating cerebral tumor is a glioma; the extracerebral neoplasm is a sarcoma. *Mouse 8*. Intracranial fibrosarcoma. Drawing of sharply circumscribed sarcoma arising in longitudinal fissure and extending extracranially. The ring of tissue around the circumference of the tumor in the upper drawing represents calvarium. *Mouse 20*. Intracranial fibrosarcoma. Drawing of circumscribed tumor in longitudinal fissure. *Mouse 30*. Intracranial fibrosarcoma. Drawing of large tumor arising in longitudinal fissure, compressing adjacent cerebral hemispheres and presenting extracranially. Distinct demarcation from the nervous parenchyma is apparent.



B.P. 1



B.P. 8



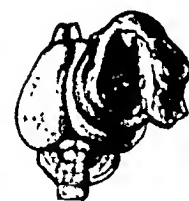
B.P. 20



B.P. 30



B.P. 20



B.P. 30



B.P. 20



B.P. 30

9

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Brain Tumors Produced with Benzpyrene

A CYTOLOGICAL STUDY OF THE TUBULAR EPITHELIUM IN ACUTE AND CHRONIC CANINE BRIGHT'S DISEASE WITH ESPECIAL REFERENCE TO THE MITOCHONDRIA*

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Though the detailed correlation of the functional and structural characteristics of the tubule of the normal nephron may be still far from adequate, it is fair to state that considerable data concerning both of these aspects of the problem of renal activity are in hand. In the case of nephrons altered by disease, these essential problems have as yet been approached only indirectly from the functional side, and in the morphological problem there is at best only information available concerning the grosser alterations of their configuration.¹

An extensive literature describes the cellular changes observed in the tubular epithelium in chronic Bright's disease. The greater part of it is concerned with what are called acute "degenerative" lesions as these are observed during varying stages of the development of the renal lesion. There are also descriptions of the regenerative processes which repair these retrogressive lesions. The cells accomplishing this restitution become in the end the only remaining functioning elements of the tubule and so must determine, or be determined by, tubular function. Concerning them, our knowledge can at present be summarized only in terms of an "atypical epithelium," for no cytological detail is available.

One of the most specific and well characterized cytological elements of the normal tubule cell is its mitochondrial apparatus. The present study attempts to describe these structures in the atypical renal epithelium of the kidney in canine Bright's disease.

The mitochondrial constituents of the kidney have been investigated experimentally in numerous acute renal lesions² but only one detailed study has been made of a chronic lesion, namely, Oliver's³ description of the mitochondria in chronic uranium nephritis. In that investigation special emphasis was placed on the relation of the mitochondrial elements to epithelial regenerative activity. As it is essential to obtain absolutely fresh tissue for satisfactory mitochondrial staining, there are no references to similar studies of human renal disease, with the exception of Fahr's⁴ description of the mitochondria in hyaline granular degeneration. The mitochondria have likewise not been examined in the naturally occurring kidney diseases of animals where fresh

* Received for publication, January 15, 1943.

material is readily available. Since the tubular epithelial changes in spontaneous renal disease of the dog closely resemble those in human renal disease, the conclusion is tenable that the morphological changes in the mitochondria of both species might be similar, if not identical. The problem in human Bright's disease may therefore be attacked indirectly by study of the animal disease.

With this object in mind, the mitochondrial elements in suitably fixed and stained kidneys of dogs with spontaneous renal lesions were examined and their appearance considered in relationship to the general morphological cellular characteristics as shown by such routine stains as hematoxylin and eosin. In the animal disease, as in human Bright's disease, the cytological changes are pronounced in the proximal convoluted tubule and the present observations are principally concerned with this portion of the nephron.

Although the mitochondria are theoretically well defined cytoplasmic constituents, their exact composition is unknown and their normal function and pathological alterations have been the subject of much controversy. The only specific method for their demonstration is supravital staining with Janus green and similar dyes. Such a procedure was impossible to apply to the dog kidney for technical reasons. It must be recognized that in fixed material other cytoplasmic materials and structures in addition to the mitochondria may take the mitochondrial stain. Exact identification of granular structures in the strict cytological sense therefore becomes difficult, if not impossible, but, in spite of this inexactness, the term mitochondria has been retained for want of any better designation. It is appreciated that conclusions based on the findings in the renal epithelium are of limited application in the general cytological problem of the mitochondria.

MATERIAL AND METHODS

The material studied was obtained from the kidneys of dogs brought to my animal hospital, and includes the acute nephroses in which only the lesions of cloudy swelling, hyaline droplet degeneration, fatty metamorphosis, vascular degeneration and necrosis were found; chronic nephroses such as amyloid contracted kidney; and inflammatory lesions, such as interstitial nephritis,⁵ and suppurative nephritis. In the dog, lipid nephrosis is unknown and glomerulonephritis is exceedingly rare.⁵ The kidneys of animals with leptospirosis⁶ also offered excellent material for the study of regeneration because of the marked renal epithelial damage that occurs in this infection. In addition, mitochondrial studies were made of the kidneys of four normal dogs.

The clinical status of renal change in each case was determined

by appropriate blood and urine examination. When the condition was apparently incurable or when the owner refused to authorize treatment, the dog was killed by intravenous injection of soluble pentobarbital, as the mitochondria are not altered by this agent.

Necropsies were performed immediately and tissues were fixed in Zenker's fluid-formaldehyde solution and in a 10 per cent solution of neutral formaldehyde U.S.P. Regaud's fluid was used to preserve tissue for mitochondrial staining. Routine sections were stained with hematoxylin and eosin, and frozen sections of formaldehyde-preserved tissue were stained for fats with Sudan III. The mitochondria were stained with Altmann's acid fuchsin and picric acid, Cowdry's acid fuchsin and methyl green, Mallory's phosphotungstic acid hematoxylin and Heidenhain's iron hematoxylin.

OBSERVATIONS

The morphological characteristics of the normal mitochondria of the dog's kidney can be summarized as follows. In the periglomerular portion of the proximal convoluted tubule the mitochondria occur as long, thin, parallel, well stained, rod-like filaments extending from the basement membrane to the supranuclear region of the cell. They are very closely packed, have a uniform thickness with rounded ends and are occasionally somewhat wavy. Many terminate just below the brush border. This appearance is best observed in tubules cut at right angles to the lumina and in longitudinal sections that pass through the axis of the tubule (Fig. 2). If the tubule section is imperfect, the rods appear as round granules of uniform size whose diameter is the same as the width of the individual rod. The terminal portion of the proximal convolution in the dog is characterized by the almost constant presence of a physiological fatty infiltration. In the fat-rich cells the mitochondria are absent or occur as a few small granules interspersed between the crowded fat droplets. In the fat-free cells of this portion of the tubule the mitochondria are similar to those of the periglomerular part of the convolution but are reduced in number. In the thin descending limb of Henle's loop the mitochondria consist of sparse numbers of pale-staining fine granules and thin, short, bacilli-like rods. These usually occupy a perinuclear position and are rarely present in the supranuclear region, which appears as a clear, cap-like, mitochondria-free space. In the thick ascending limb of the loop and the distal convoluted tubule the mitochondria occur as short, stout, homogeneous rods extending from the basement membrane to the supranuclear region. They are paler staining and less densely packed than those in the periglomerular portion of the proximal convolution.

In the collecting tubules the mitochondria are exceedingly sparse and consist of pale-staining, very short, thin, delicate rods and fine granules indiscriminately scattered in the cytoplasm.

The mitochondrial changes in the various renal lesions to be described can be conveniently classified by considering the types of epithelial cells in which they occur. The categories of epithelia that develop in a chronic renal lesion have been previously enumerated⁷ and this classification will be used as a basis for the morphological descriptions of the mitochondria.

1. Persisting Epithelium of the Original Renal Type

(a) *In Cells Normal in Structure.* The mitochondria showed no change from the normal picture in cells normal in structure.

(b) *In Cells Undergoing Acute Regressive Changes* such as cloudy swelling, hyaline droplet accumulation, fatty metamorphosis, vacuolar degeneration and necrosis. A variety of mitochondrial alterations occurred in these degenerative states. In some the mitochondria had lost their long filamentous appearance and fragmented into occasional small fine rods and numerous fine granules whose diameter did not exceed the width of an individual rod. Such cells were frequently swollen and presented the usual characteristics of cloudy swelling (Fig. 3). In other cases the granules were deeply staining, coarse and usually globular, with considerable irregular variation in size and a greater diameter than the width of the normal rod (Figs. 4 and 5). These cells, in hematoxylin and eosin preparations, indicated a more advanced stage of cloudy swelling with large, coarse, eosinophilic granules in a greatly swollen cytoplasm. Granules of all these types diffusely filled the cytoplasm, showing no definite cellular orientation. Material having all the staining characteristics of mitochondria also assumed "droplet" form. The droplets, large, deeply staining, spherical and with smooth contours, varied considerably in size and were often as large as a nucleus (Fig. 6). They likewise evidenced no definite cellular distribution and occasionally occurred in the tubular lumina. The droplets appeared in sections stained with hematoxylin and eosin as typical "colloid droplet" formations. In cells that were definitely necrotic the granules were agglutinated into large, irregular masses of mitochondrial material that were more heavily concentrated in the basal or distal portions of the cell and which frequently fused with similar conglomerated material in adjacent cells. In vacuolar degeneration the vacuoles themselves contained no mitochondrial material although fine granules and short rods were commonly present in the intervacuolar cytoplasm (Fig. 7). Mitochondria were usually absent

in degenerating cells containing fat similar in appearance to that seen in the physiological infiltration of the cells of the terminal proximal convolutions.

(c) *In Epithelium Undergoing Progressive (Hypertrophic or Hyperplastic) Change.* The filamentous mitochondria of the hypertrophic periglomerular proximal convolutions in chronic renal lesions were definitely lengthened although there was little, if any, increase in their width (Fig. 8). In striking contrast there was no increase of the normally sparse number of mitochondria in the hyperplastic collecting tubules (Fig. 9).

(d) *Hypertrophic Cells in Which Degenerative Change Has Secondarily Occurred.* The mitochondrial alterations, namely, fine and coarse granules, droplets and agglutinated masses, in these large cells were similar to those seen in normal cells undergoing regressive changes (Fig. 10).

2. Newly Formed Regenerated Epithelium

Among the kidneys in all of the conditions studied there were found all stages in the regeneration of epithelium. Numerous mitotic figures were present and there was great variation in the amount of mitochondrial material in these cells. Some cells in mitosis contained large numbers of granular mitochondria and in others these elements were completely absent. In addition, dividing cells could be found whose mitochondrial content varied in a quantitative manner between these extremes, so that a series could be arranged beginning with those containing many granules and continuing with cells almost completely absent (Fig. 1). The majority of the mitotic figures showed abnormalities in the arrangement and distribution of their chromatin. Hyperchromatic (Fig. 1, h and j) and hypochromatic (Fig. 1, d) figures were common, as well as pyknosis (Fig. 1, c) and rhexis of chromosomes (Fig. 1, e and i), as these terms are used by Politzer.⁸ Gigantism also was observed even in the earliest prophases of division (Fig. 1, a and b).

As the cells of tubules with regenerated epithelium do not show a constant fixed cytological type, various stages in the maturity of the new-formed cells could be identified. Tubules with early regeneration of epithelium often consisted of a bare membrana propria with several large, oval, hyperchromatic nuclei that projected into the lumen and which were covered with a thin, indistinct cytoplasmic layer. In these cells mitochondria were completely absent or at best only an occasional granule was present (Fig. 11). The epithelium of other tubules with more advanced regenerative changes consisted of many large nuclei lying in an undifferentiated, cytoplasmic, syncytial mass. These often

assumed the appearance of "giant cells" and consisted of from 6 to 15 nuclei in a mass of cytoplasm that extended into and more or less filled the tubular lumen. In the syncytial masses and "giant cell" complexes, only a sparse number of small, granular mitochondria were present and in some of these tubules, mitochondria were completely absent (Figs. 12 and 13). The cytoplasm of these cells, however, usually contained a fine, dust-like material that took the mitochondrial stain very faintly.

In kidneys with acute degenerative lesions but in which there was no interstitial proliferation of connective tissue, the new cells gradually took on, with the passage of time, the appearance of the adult epithelium and eventually could not be differentiated from the original structures. In these cells, the nuclei decreased in size, lost their vesicular appearance and regained their usual polarity in relation to the tubular lumen; the cytoplasm became more distinct and evidenced cellular differentiation. In addition, there was an early development of fine granules and short rods until the mitochondria characteristic of the original cell type appeared (Figs. 14 and 15).

In kidneys with chronic Bright's disease, namely, amyloid contracted kidney and chronic interstitial nephritis, the atypical regenerated epithelium in tubules in areas of interstitial fibrosis persisted as such and never regained the structure of the normal adult cell, so that these distorted tubules were lined with an irregular layer of epithelium often several cells thick, whose oval nuclei showed no orientation in regard to the axis of the tubule. In the atypical cells of these tubules the mitochondria never assumed their normal rod-like structure but consisted of scattered small granules in sparse numbers. This persistence of large groups of distorted tubules with atypical epithelium and scanty mitochondria was noted even in those damaged kidneys where, judging by the clinical evidence of adequate renal elimination, functional compensation had occurred. In such instances the convoluted tubules outside the scarred areas were hypertrophied and hyperplastic and possessed an entirely normal mitochondrial pattern.⁷

3. Epithelium of the Preceding Categories That Has Undergone Simple Atrophy

The mitochondria of atrophic epithelium, whether compressed and distorted, or flattened, were similar. They consisted of small, fine, pale-staining granules and short, thin rods that were often more heavily concentrated around the nucleus (Fig. 16). Even in tubules whose lumina were obliterated and whose membranae propriae had blended with the surrounding connective tissue, the epithelial cells contained

few persisting mitochondrial granules. These atrophic cells rarely showed degenerative change, such as large granule forms, droplets, or agglutinated masses.

DISCUSSION

In general, it may be said that the morphology of the mitochondria is apparently correlated with the general histological appearance of the tubular epithelium, so that if one is familiar by means of special stains with the mitochondrial changes peculiar to the various epithelial alterations, it is possible to predict with a fair degree of certainty the microscopical mitochondrial morphology by the study of even a simple routine preparation.

As has been stated, most of the experimental investigations relating to the renal mitochondria have been concerned with their reactions in acute renal lesions, and these have been adequately summarized by Cowdry.² The mitochondrial changes in the degenerative epithelial lesions (Figs. 3 to 7) in my series of spontaneous renal disease closely approximate those that have been previously described in experimental acute renal damage.

The derivation of the hyaline droplets which occur in the renal epithelium in a variety of conditions deserves special comment as these have been interpreted as being of exogenous origin by some, *i.e.*, as protein absorbed from the tubular lumen, and of endogenous origin, arising from intracellular mitochondrial material, by others. In my material, the hyaline droplets have the tinctorial properties of mitochondrial substance and in the same cell all forms, from small granules to large droplets, may be seen together (Fig. 6). The histological data suggest, therefore, that the droplets consist, in part at least, of mitochondrial material, or that this material enters into their composition and that droplets may arise by swelling, solution and fusion of the granules.

According to Cowdry,² an increase of mitochondrial material is relatively rare and has been reported in the thyroid, in the islets of Langerhans during diabetes, in the kidney following the administration of phloridzin, and in actively regenerating muscle and cartilage cells. In the cases of compensated chronic nephritis of this series the mitochondrial rods of the hypertrophic periglomerular proximal tubules are definitely lengthened with no increase in width, so that the total amount of mitochondrial material within each cell is augmented (Fig. 8). This increase becomes considerable when the exact mathematical dimensions, which have been calculated from measurements of microdissected specimens, are considered,⁸ inasmuch as the hypertrophic

proximal convolution is not only wider and has a greater volume but may be almost twice as long as the normal.

Addis and Oliver⁹ summarized the facts concerning the regenerative processes of the tubular epithelium in human renal disease. Essentially, the reparative epithelial changes are similar in Bright's disease of man and dog and in experimental investigations with bichromate salts, corrosive sublimate, or uranium nitrate. The epithelium which has escaped the destructive action proliferates by mitosis and possibly by amitosis. In my material, abnormal mitotic figures were the rule. The series which may be set up of cells with mitotic figures, some with many granules and others with none, apparently indicates a continuing decrease with eventual disappearance of the mitochondria during the course of abnormal renal regenerative activity (Fig. 1). The mechanism of this phenomenon may be as follows: With the first division of the cell the number of mitochondria distributed to each daughter cell is approximately half that of the mother cell and with each rapidly succeeding division the mitochondria are further reduced so that the definitive regenerated cell contains very few, if any, granules. Although the studies of Lewis and Lewis¹⁰ of tissue cultures show wide variations in the amount or number of the mitochondria in proliferating chick embryo cells, Cowdry,¹¹ also working with chick embryos, came to the general conclusion that the number, shape and arrangement of mitochondria during mitotic division are essentially the same as in nondividing cells. This discrepancy with what I have described can be explained by the fact that in my material regenerative activity followed definite cellular injury, as contrasted with the mitotic activity of the normal cells of the chick embryo, and that in the chronic lesions it proceeded under the conditions of a chronic disease which precluded the re-establishment of the original normal status.

The finely granular, extremely pale-staining, dust-like material (Figs. 12 and 13) in the regenerated epithelium can be interpreted either as representing further fragmentation of the persisting mitochondria or as an early stage in the formation of new mitochondrial substance. The former supposition appears more likely as occasional tubules were seen lined in part by epithelium of mature appearance in which mitochondrial substance was completely absent, suggesting that this granular material had gone into solution and disappeared. This action might be considered as an indication of continuing cellular damage exerted by the toxic or infectious agent responsible for the initial renal lesion, and that the agent was still active in its nephrotoxic effect on the newly formed immature epithelium.

For years, cytologists have been interested in the doctrine of mito-

chondrial continuity,¹² in respect to whether the mitochondria in proliferating cells arise in the cytoplasm *de novo*, or only through multiplication by division of pre-existing units. While the evidence presented here is far from incontrovertible, it seemed more likely that the mitochondria of the latter generations of regenerated cells arose in the cytoplasm *de novo* as a result of the cells' metabolism and not through division of pre-existing units.

It is generally known, as Thorel¹³ first demonstrated, that in many epithelial degenerations in the kidney the regenerated cells, though at first of an embryonic type, later assume a form which differs in no degree from the adult type of the original renal cells. However, the early proliferation of connective tissue in chronic nephritis³ apparently prevents the regenerated epithelium from reaching maturity and thus the epithelium never regains the mitochondrial characteristics of the adult cell. Addis and Oliver⁹ suggested that a similar mechanism may operate in the kidneys of man with chronic Bright's disease. Certainly in chronic canine Bright's disease, the "atypical" epithelium has a permanently reduced mitochondrial content, and in this respect closely resembles the earlier generations of immature epithelium that are found in the repair of transient degenerative renal lesions. Whether this inability of differentiation to the original cell type is due to the persisting toxic effect that produced the original retrogressive change in the epithelium or to abnormalities in environment and nutrition that have developed in the altered renal tissue during the course of the chronic disease is a speculative question. This problem is further complicated by the possibility of a genetic factor, for it is certain that the atypical epithelium arises in large part by atypical karyokinesis.

Architecturally, *i.e.*, in the configuration and number of their nephrons, the clinically compensated and decompensated kidneys of chronic canine Bright's disease may be similar, although in the tubules of the latter are found characteristic cellular structural alterations. In a previous study⁷ it has been shown that from the morphological viewpoint, the mitochondrial elements of the proximal convolution are essentially normal in the adequately compensated organ and considerably disarranged in the decompensated kidney. The difficulty of ascertaining the functional state of a kidney by examination of the routinely stained section is well known. A definite indication can be obtained as to the state of compensation or decompensation in chronic canine Bright's disease, however, by the application of stains for the mitochondrial apparatus. This is true, of course, only when fresh tissue is immediately preserved in suitable fixatives, for poor or late fixation simulates the same disintegration of the mitochondrial elements that

is found in the decompensated kidney associated with bona fide disease processes.

The tubule of the nephron in chronic Bright's disease is lined with an epithelium that differs from the normal not only in its general cellular characteristics but equally so in the intimate cytological structures of its cells. Speculations as to the existence of possible correlated disturbances of its function would be premature, but if one accepts the general proposition of correlation of structure and function, associated disarrangements of the latter might be suspected.

CONCLUSIONS

1. A cytological study of the kidneys of dogs with spontaneous acute and chronic Bright's disease indicates specific alterations of the mitochondrial elements in the tubular epithelium, associated with the regressive, progressive and regenerative changes.

2. The morphological mitochondrial pattern is closely correlated with the general histological appearance of the tubular epithelium as determined by routine stains.

3. As the general morphological aspect of the tubular epithelial changes in human and canine Bright's disease are essentially the same, it is suggested that the mitochondrial alterations in both species may be similar.

I wish to thank Dr. Jean Oliver for his guidance and aid in this work.

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[Illustrations follow]

DESCRIPTION OF PLATES

KEY TO PLATE 118

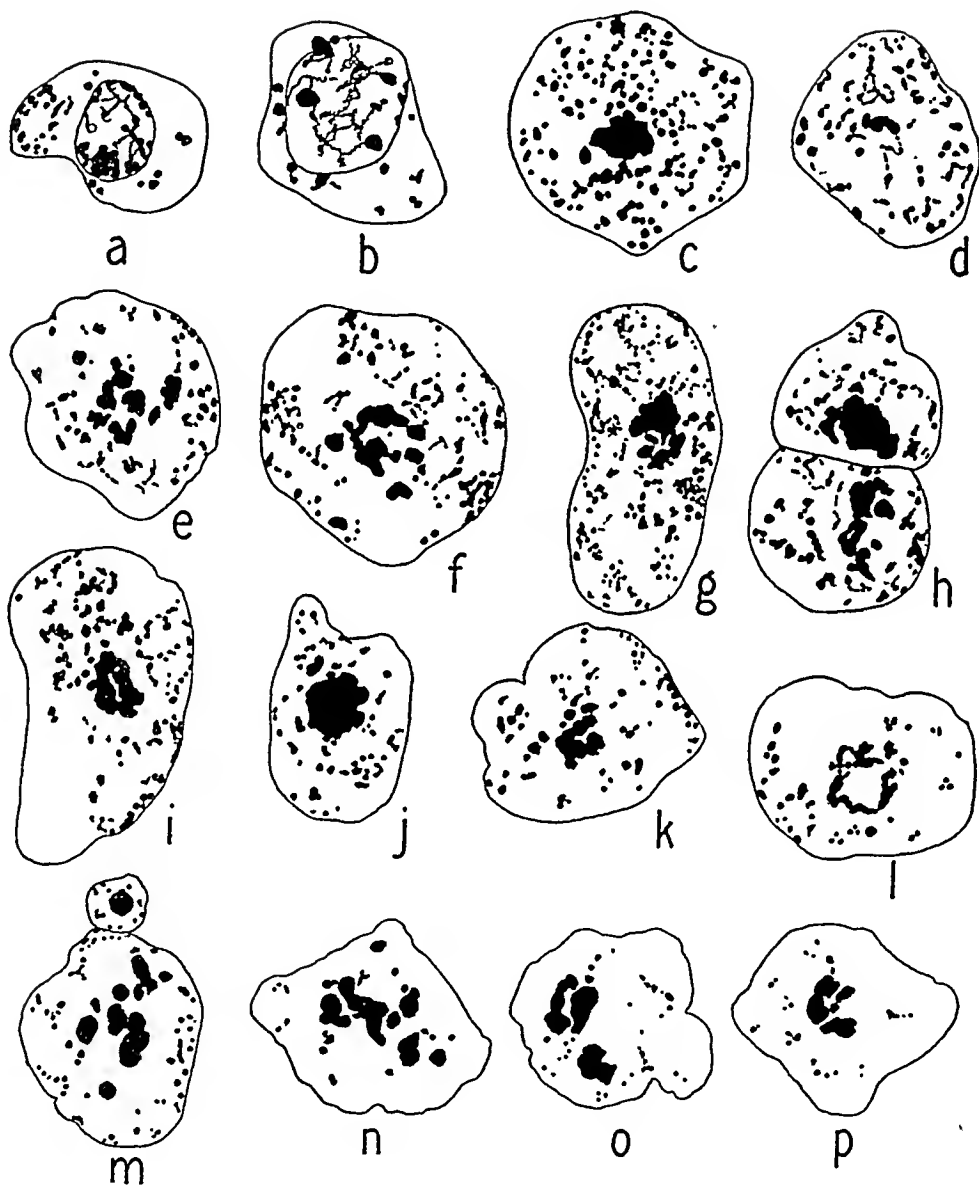


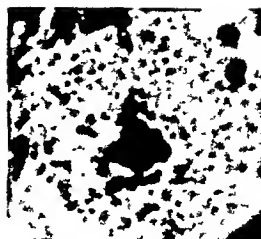
FIG. 1. a to p, mitotic figures from regenerating renal epithelium in canine Bright's disease. a, c, d, f, j and p, chronic interstitial nephritis, iron hematoxylin stain; b, e, k, m and d, subacute interstitial nephritis, Mallory's phosphotungstic acid hematoxylin stain; g, h, i, l, n and p, subacute nephrosis, iron hematoxylin stain. $\times 1500$.



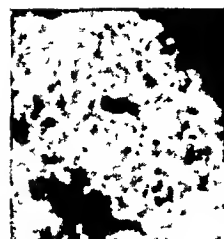
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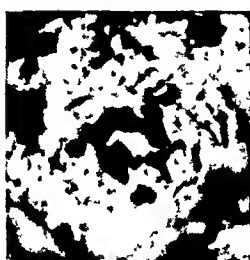
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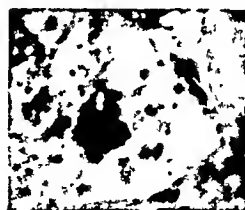
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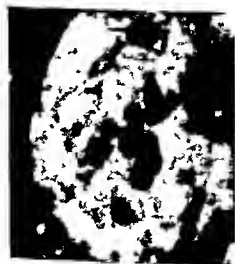
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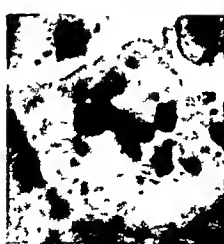
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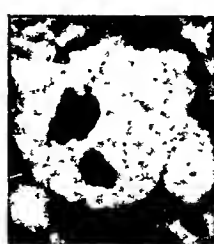
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PLATE 119

FIG. 2. Case 709. Chronic interstitial nephritis. Cross section of proximal convolution in the periglomerular mass. The persisting tubular epithelium is entirely normal. The mitochondria appear as long, densely packed, filamentous rods. Iron hematoxylin stain. $\times 1000$.

FIG. 3. Case 887. Chronic interstitial nephritis. A periglomerular proximal convolution shows various degenerative disarrangements in the mitochondria. At the left, dissolution of the mitochondrial rods and vacuole formation are seen; in the middle portions the filaments have broken up into fine granules; at the right, these granules are agglutinated into an irregular mass. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

FIG. 4. Case 5935. Leptospirosis. A longitudinal section of a periglomerular proximal tubule in which the normal rod structure has disintegrated and the cytoplasm is filled with large, coarse, deeply staining granular mitochondria. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

FIG. 5. Case 5592. Acute interstitial nephritis. Sections of two periglomerular proximal convolutions with advanced regressive changes. Coarse granular mitochondria are present in fewer numbers as compared to Figure 4. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

FIG. 6. Case 887. Amyloid contracted kidney. A cross section of a periglomerular proximal convolution from the same case as shown in Figure 10. The cytoplasm and tubular lumen contain numerous smaller and larger droplets of material with the staining reactions of mitochondria. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

FIG. 7. Case 6457. Leptospirosis. Cross sections of periglomerular proximal convolutions showing the marked cytoplasmic vacuolation and sparse mitochondrial granules in the intervacuolar cytoplasm. Mallory's phosphotungstic hematoxylin stain. $\times 800$.

FIG. 8. Case 709. Chronic interstitial nephritis. A greatly hypertrophied proximal convolution from the periglomerular mass of the same kidney as shown in Figure 2. Except for increase in size, the cellular configurations are entirely normal. The mitochondria are correspondingly increased in number and in length. Figures 2 and 8 are shown at the same magnification and are therefore directly comparable. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

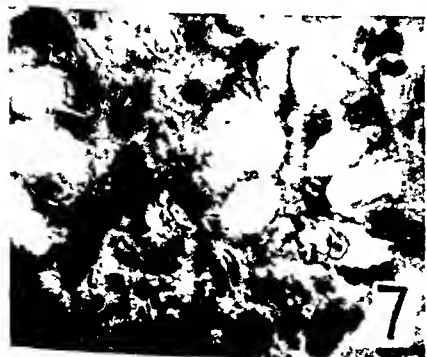
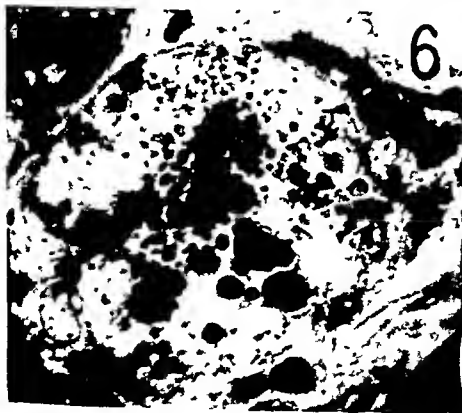
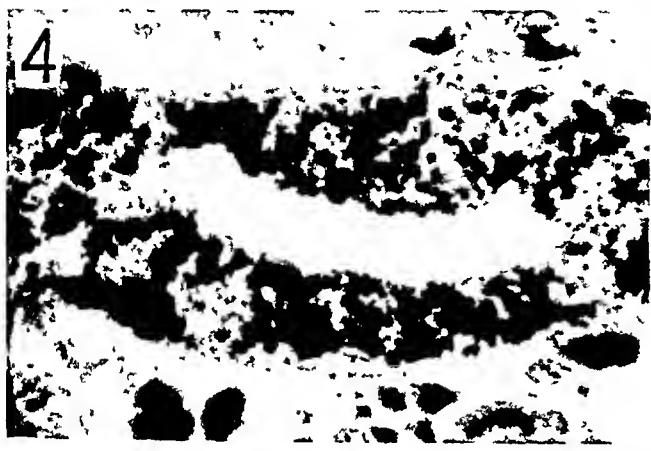
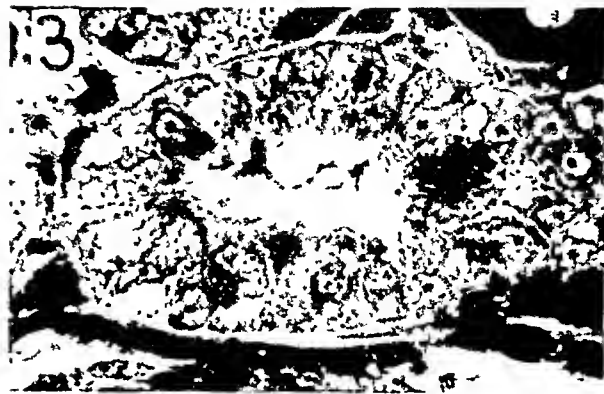
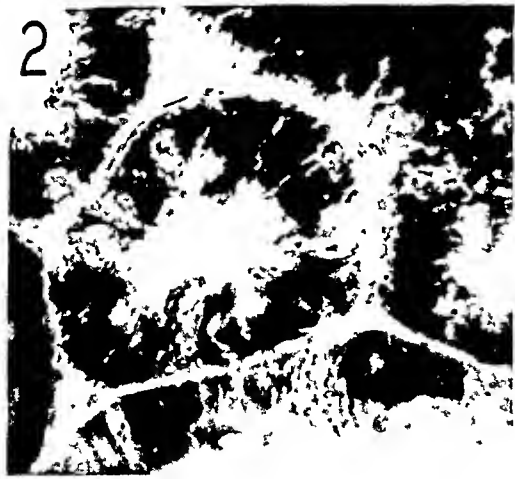


PLATE 120

FIG. 9. Case 709. Section of a markedly hypertrophic collecting tubule from the same kidney as shown in Figure 8. In these large epithelial cells there is no increase in the mitochondria, which are originally scanty in these tubules. Iron hematoxylin stain. $\times 1000$.

FIG. 10. Case 887. Amyloid contracted kidney. Cross section of a hypertrophic periglomerular proximal convolution in which the normal rods are absent and the mitochondria are fragmented into fine, paler staining granules. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

FIG. 11. Case 5935. Leptospirosis. An atypical tubule with several regenerated cells, in one of which there is a mitotic figure. The nuclei are large and oval and the cytoplasm indefinite and scanty. There is practically complete absence of mitochondrial granules, although finely granular, dust-like material is present. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

FIG. 12. Case 5935. Leptospirosis. A later developmental stage in which the cells assume a giant-cell-like appearance. Very few pale-staining mitochondrial granules are present in addition to the dust-like granular material that very faintly takes the mitochondrial stain. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.

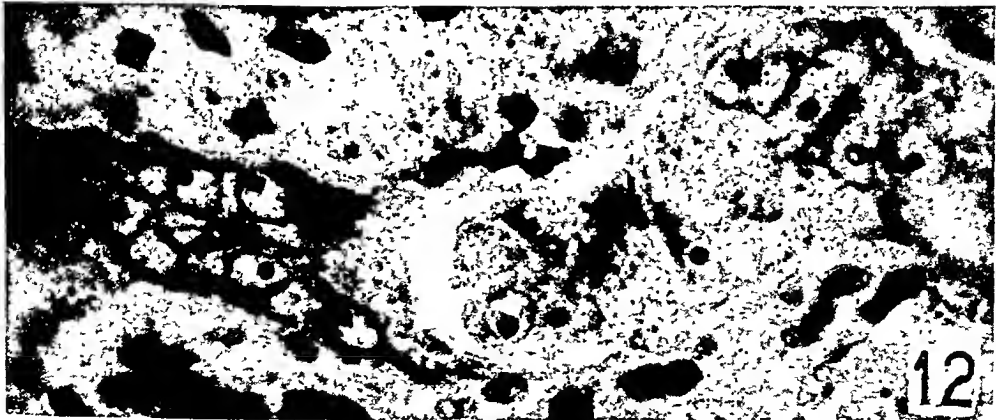
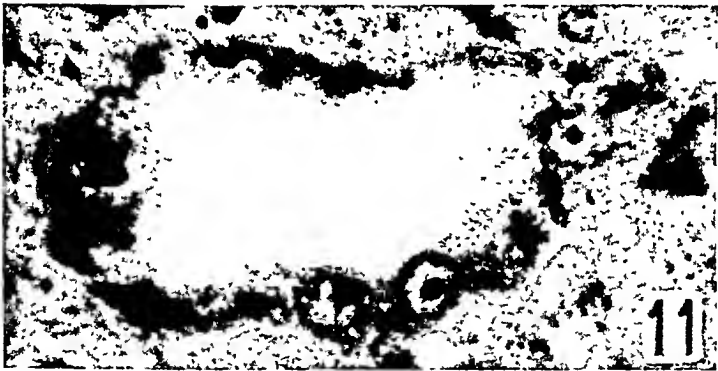
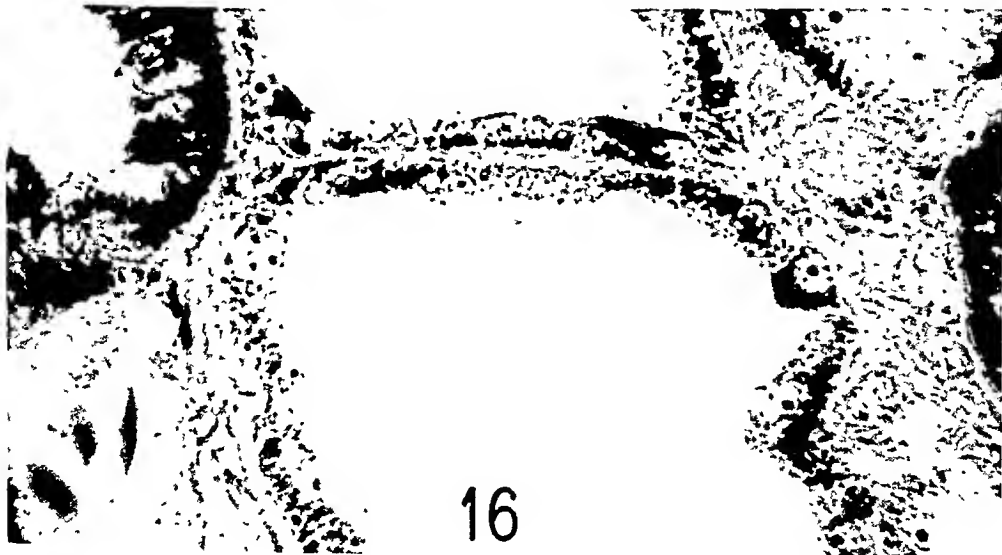
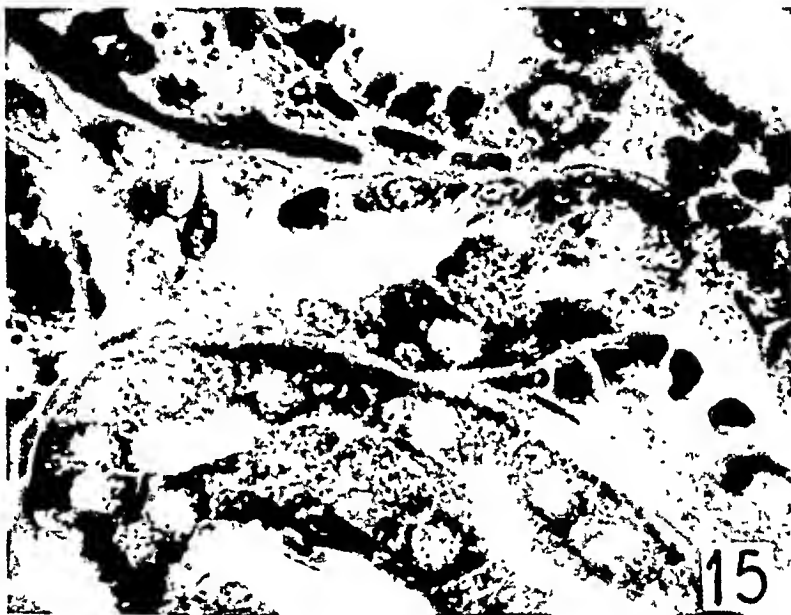


PLATE 121

- FIG. 13. Case 5935. Leptospirosis. Another tubule from the same kidney as shown in Figure 12, in which there are a greater number of mitochondrial granules and more dust-like, finely granular material. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.
- FIG. 14. Case 5592. Acute interstitial nephritis. Lying diagonally are longitudinal sections of two tubules, the cells of which are beginning to assume a more adult appearance. In these there is a perinuclear distribution of small, granular mitochondria while the remaining immature cells are lacking granules. Compare with the deep-staining, coarse granules of the degenerated epithelium in the right lower corner. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.
- FIG. 15. Case 5592. Acute interstitial nephritis. The cells in the left portion of the upper middle tubule are similar to those of Figure 14, with only a few cells showing perinuclear granular mitochondria. In the portion of the same tubule to the right the cytoplasm of the cells of more mature configuration is markedly increased and contains numerous closely packed granules. These latter cells are considered a later stage in epithelial regeneration. The tubule below is completely lined by similar cells. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.
- FIG. 16. Case 200. Chronic interstitial nephritis. Widely dilated tubules with flattened atrophic epithelium in which fine granules and short rods are present. Mallory's phosphotungstic acid hematoxylin stain. $\times 1000$.



Bloom

Tubular Epithelium in Canine Bright's Disease



NECROTIZING ARTERITIS IN DOGS RELATED TO DIET AND RENAL INSUFFICIENCY

V. EVIDENCE FOR A DIETARY FACTOR*

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In recent publications from this laboratory¹⁻⁴ it has been shown that acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, pulmonary artery, endocardium of the left auricle) occur with regularity following the production of renal insufficiency in dogs that have been fed a standard low protein diet. The manner in which renal insufficiency was produced—uranium nitrate, mercuric chloride, or bilateral nephrectomy—did not influence the incidence of these arterial lesions. Only two factors seem to be necessary for their production: (1) a factor associated with renal insufficiency, and (2) a dietary factor. The first factor has received ample confirmation from the work of Winternitz and his co-workers^{5, 6} who have produced somewhat similar lesions in dogs by injecting various extracts of different organs in dogs with both kidneys removed, by ligation of both renal arteries and by total and subtotal obstruction of both ureters. The work of Goldblatt,⁷ showing that renal ischemia severe enough to cause renal insufficiency leads not only to hypertension but also to a necrotizing arteriolitis such as is encountered in human cases of malignant hypertension, also confirms the factor associated with renal insufficiency. Neither of these groups of workers, however, has found it necessary to control the diet of its experimental animals. In our experience to date, diet seems to play a definite rôle. It is the purpose of this paper to describe in detail the preparation and feeding of the standard diet and to present the data that incline us toward the belief that a dietary factor is fundamentally associated with the lesions.

METHODS

All of the dogs were healthy adult mongrels. Pups and obviously old dogs were excluded. They were brought into the kennel from routine local sources and were kept in "isolation" for 2 weeks before being placed in the main dog room. Both during the 2 weeks' period of "isolation" and during their subsequent stay in the main dog room they were kept in individual cages (on shavings that were changed three

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times per week). They had free access to water at all times and, unless otherwise indicated, they were fed the regular kennel ration which has varied from time to time. The major portion of this ration has at times consisted of cooked table scraps selected from refuse at the University dining hall cafeteria. At other times the kennel ration has consisted chiefly of uncooked bones with adherent fat and meat. Occasional feedings of Purina dog chow have been used when other food was not available. Temperature and humidity in the animal quarters are controlled throughout the year. During the course of these experiments there have been no outbreaks of distemper or other known infectious diseases.

The principal variables in the experiments reported in this and the following papers are four in number: (1) diet, (2) renal insufficiency, (3) "plasma alteration" and (4) time. The methods for controlling these variables have been described in previous publications.¹⁻⁴ Since the emphasis in this paper is on diet, the preparation and feeding of the standard diet is given in detail.

The "standard diet" fed most of the animals in the experimental group consisted of: calves' liver (raw wet weight), 32 parts; cane sugar, 25 parts; corn starch, 25 parts; butter, 12 parts; and cod liver oil, 6 parts. One gm. of McCollum-Simmonds salt mixture⁸ and 5 gm. of kaolin were thoroughly mixed with each day's diet. Enough tomato juice was added to make a paste of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per Kg. per day. Essentially the diet is a low protein diet with 7 per cent of its caloric value derived from protein, 50 per cent from carbohydrate and 43 per cent from fat.

In actual practice the total number of dog diets for about 1 week (5 to 10 days extremes) was calculated and a batch of food to supply this number of kilogram-dog-days was prepared as follows:

Cane sugar	19.2 parts per 100
Corn starch	19.2 parts per 100
Kaolin	4.0 parts per 100
Salt mixture	0.8 parts per 100

After this dry material was thoroughly mixed, the following were added:

Liver (finely ground in meat chopper)	24.0 parts per 100
Tomato juice	18.4 parts per 100

Again the mixture was stirred and the following added:

Cod liver oil (commercial)	4.8 parts per 100
Butter (melted but not boiled)	9.6 parts per 100

These last two were thoroughly stirred into the mixture to form a homogeneous paste which was kept in the ice box at 36° to 38° C.

Each day's food for each dog fed the standard diet was weighed out into individual pans and placed in the cage, customarily between 2 and 4 p.m. Any food left the following morning was weighed and complete records were kept of dietary consumption. Rarely a dog (less than 1 out of 10) refused to eat the diet, hence all dogs were placed on this standard diet for a few days (usually 3) to determine that the diet would be eaten before control blood-level studies were obtained. Dogs made hypoproteinemic were fasted for 1 week before being returned to the standard diet which was fed while the daily exchanges were being made. There was no interruption of the diet in any other dogs.

"Plasma alteration," when employed, depended on one of three techniques: (1) induction of hypoproteinemia, (2) plasma injections and (3) citrate injections. Hypoproteinemia was produced by plasmapheresis; hyperproteinemia, by repeated intravenous injections of homologous plasma (the anticoagulant was tri-sodium citrate, 1.25 cc. of a saturated aqueous solution—about 0.8 gm. per 100 cc. of donor's blood); and citrate injections consisted of repeated intravenous injections of an equivalent amount of tri-sodium citrate added to 100 cc. of 0.9 per cent sodium chloride.

Renal insufficiency was produced in one of three ways: (1) uranium nitrate (uranyl nitrate, Merck, lot S-7497) injected into the subcutaneous tissues of the groin in 0.5 per cent aqueous solution; (2) mercuric chloride (Baker's analyzed, lot 6939) injected into the external jugular vein in 0.1 per cent aqueous solution; and (3) bilateral nephrectomy performed with aseptic technic.

Following the production of renal insufficiency the dogs usually ceased eating from 1 to 10 days later and 2 to 10 days before they died. The dogs that survived renal injury varied to some extent, but most of them continued to eat the diet each day of their survival period. The data related to dietary consumption *after* renal insufficiency have been analyzed, but no significant differences related to this factor could be detected; the important factor seemed to be the duration on the standard diet *before* renal insufficiency was produced.

Nonprotein nitrogen was determined by duplicate micro-Kjeldahl analysis. The protein precipitant used in separating protein from nonprotein nitrogen was 10 per cent trichloroacetic acid.

The experimental animals were kept under close observation, were sacrificed with ether if they were obviously moribund and were necropsied promptly after death. Routine sections were taken from the

following sites: mucous membranes of mouth, injection site in groin, heart [three sections: (a) left ventricle, mitral valve and left auricle—this section included the circumflex branch of the left coronary artery; (b) right ventricle, pulmonic valve and pulmonary artery; and (c) interventricular septum, aortic valve and base of aorta], aorta and any of its larger branches that showed gross change, lungs (at least one section from the hilus region), spleen, liver, gallbladder, pancreas, adrenal, kidneys, bladder, prostate and testis in males, ovary and uterus in females, stomach, small intestine (usually including a Peyer's patch), colon, mesenteric lymph nodes, thymus, thyroid and parathyroid, hypophysis, brain, eye, rib, and femoral bone marrow. The procedure was varied to include any gross lesions. Tissue from these sites was fixed in a 4 per cent solution of formaldehyde and in Zenker's solution. The Zenker's fixed tissues were sectioned at $7\ \mu$ and stained routinely with hematoxylin and eosin. Special stains were used in selected cases.

EXPERIMENTAL OBSERVATIONS

The data which form the basis for this paper have been abstracted from two series of experiments: (1) a series primarily for a study of arterial lesions in which most of the dogs were fed the "standard diet," and (2) a control series (an outgrowth from the first series) which was designed to study the effect of sodium citrate on heavy metal injury and in which practically all of the dogs were fed the "kennel diet." Most of the control data come from this second series of experiments.⁹ In Table 1 of the following paper¹⁰ the data on the first series of dogs are given in some detail, and the subgrouping of these dogs—by diet, period on diet, method of production of renal injury, and "plasma alteration"—has been indicated by numbers in the last column. These subgroup numbers appear in some of the tables of this paper. This method of presentation of the data enables one to check illustrative examples of the type of experiment referred to in the tables of this paper. The difference between the numbers listed in the latter and the number of examples given in Table 1 of the following paper¹⁰ represents the number of dogs in the "control" or "sodium citrate" series.

Table 1 groups all of the dogs together to substantiate the effect of diet upon the incidence of arterial lesions. Of the 97 dogs which consumed an unknown amount of a variable *kennel diet* for an indefinite period of time before being subjected to renal insufficiency by uranium nitrate or mercuric chloride, only 5, or 5.2 per cent, developed arterial lesions. The incidence of arterial lesions was about four times as great

TABLE I
*Influence of Diet, Method of Production of Renal Insufficiency, and "Plasma Alteration"
 on the Incidence of Arterial Lesions*

Diet	Renal insufficiency produced by	"Plasma alteration"	Number of dogs	Number with arterial lesions	Per cent positive	Subgroup number*
Kennel	Uranium nitrate	None	22	0	0	1
		Citrate injections	49	2	4	2
	Mercuric chloride	None	11	1	9	3
		Citrate injections	15	2	13	4
	Total		97	5	5	
"Standard"	Uranium nitrate	None (4 wks. of diet)	5	0	0	5
		None (10 wks. of diet)	5	5	100	6
		Citrate injections	3	0	0	7
		Plasma injections	6	6	100	8
		Hypoproteinemia	2	2	100	9
	Mercuric chloride	None (6 wks. of diet)	3	2	67	10
		Plasma injections	3	2	67	11
	Bilateral nephrectomy	None (20 wks. of diet)	3	2	67	12
		Plasma injections	3	3	100	13
	Total		33	22	67	
Lean Meat	Uranium nitrate	Plasma injections	3	0	0	14
	Mercuric chloride	Plasma injections	1	0	0	15
	Total		4	0	0	

* These subgroup numbers correspond with those in Table I of the following paper.¹⁰

in the groups injected with mercuric chloride as in the groups injected with uranium nitrate; and four of the five instances of arterial lesions occurred in dogs that had received citrate injections. A much larger series of animals would be necessary to establish the significance of these statements; but it is significant that only 5 of the entire group developed arterial lesions, and it is worth noting that of the 2 dogs with lesions in subgroup 2 (uranium nitrate plus citrate injections) both had evidence of chronic renal damage—in one, chronic glomerulonephritis, and in the other chronic pyelonephritis. Since this is the first time that we have referred to arterial lesions in control dogs it seems advisable to document them.

A brief summary of the clinical data on these 5 dogs is given in Table II and the anatomical distribution of the lesions is listed in Table III. Typical lesions are illustrated in Figures 1 to 4. As near as I can tell, these lesions are identical with those described and illustrated in previous publications. The first change is edema and swelling of the intercellular tissue of the large elastic arteries (ascending aorta, pulmonary artery, endocardium of the left auricle) usually

TABLE II
Summary of Experimental Data on "Control" Dogs with Arterial Lesions

Dog number	Sex	Estimated age (yrs.)	Body weight (Kg.)	Diet*	Renal† injury produced by	Survival interval (days)	Terminal N. P. N. (mg. per 100 cc.)	Remarks
40-72	F	4	24.8	K. D.	Hg 2.0	4	330	Dog used as donor for 18 months
40-88	M	1	17.5	P. D. C.	Hg 3.0	10	523	
B-31			19.2	K. D.	Hg 3.0	10	320	Chronic pyelonephritis Chronic glomerulonephritis
K-20	M		11.5	K. D.	U 5.0	14	605	
K-42		12	22.0	K. D.	U 5.0	11	292	

* P.D.C. = Purina dog chow; K.D. = kennel diet.

† Hg 3.0 = mercuric chloride, 3.0 mg./Kg.; U 5.0 = uranium nitrate, 5.0 mg./Kg.

TABLE III
*Anatomical Distribution of Arterial Lesions **

Dog number	Ascending aorta	Coronary arteries	Left auricle	Pulmonary artery	Other arteries
40-72			+	+++†	Acute periarteritis of myocardial arterioles (+)† Mesenteric (+) and left subclavian (+)
40-88		+	†	+	
B-31	++	+	+++	+++	
K-20	+	+	++	++	
K-42	†	+	+++	+++	

* The lesions have been graded as follows: +++ = gross lesion over 1 cm. in maximum diameter; ++ = gross lesion less than 1 cm. in maximum diameter; + = lesion discovered in microscopical sections.

† Microscopical illustration.

near the internal elastic membrane. This is rapidly followed by outpouring of fibrin, red blood cells and leukocytes. The predominant recognizable leukocyte is the polymorphonuclear neutrophil, but the majority of the leukocytes are most accurately described as "cells with distorted nuclei." Nuclear fragmentation and other necrotic changes are marked. The lesion remains confined beneath intact endothelium until it has attained considerable size, then it may break through the endothelial covering with ensuing thrombus formation. In the advanced lesions the elastic framework is fragmented and partially lysed, but in the early lesions the elastic tissue shows only swelling. Sometimes calcium is deposited in the lesions. For further details the reader is referred to previous publications.¹⁻⁴

In contrast to the control group on kennel diet is the group of 33 dogs which were fed the "*standard diet*" for 4 weeks or longer before

TABLE IV
*Effect of Diet upon the Incidence of Arterial Lesions
following Renal Injury*

Dietary group	Number of animals	Number with arterial lesions	Per cent positive
Standard diet	25	22	88.0
Kennel diet	97	5	5.2

a comparable degree of renal injury was produced (by uranium nitrate in 21, by mercuric chloride in 6, and by bilateral nephrectomy in 6). Typical arterial lesions occurred in 22 of this group, an incidence of 67 per cent (Table 1). If the 5 dogs which were fed the standard diet for only 4 weeks (subgroup 5) and the 3 dogs which received citrate injections (subgroup 7) are excluded, the incidence becomes 22 of 25 positive, or 88 per cent (Table IV). The lesions both grossly and histologically have always corresponded to the interval between the production of renal insufficiency and death (4 days to 11 months). In addition to the usual acute necrotizing lesions which have been described and illustrated in previous publications¹⁻⁴ and which are essentially identical to those described, there have been 2 dogs with aneurysms (both near the mouth of the innominate artery) and 4 dogs with healing or healed lesions. Of the 3 dogs which did not show lesions, 1 (dog 40-83, subgroup 12) died 3 days after bilateral nephrectomy and showed early changes but no definite necrosis, another (dog 42-2, subgroup 10) was fed the standard diet for only 6 weeks before renal injury was produced, and for the third (dog 41-89, subgroup 11) there were no circumstances which might explain the lack of lesions.

The general statement that with the same degree of renal injury

about 90 per cent of the dogs on the standard diet developed arterial lesions while the incidence of similar lesions in dogs fed the regular kennel ration was about 5 per cent holds; but the detailed data must be analyzed further in order to correlate them with previously published results.¹⁻⁴ Several facts emerge from such an analysis.

First, the duration of maintenance on the standard diet before renal insufficiency was produced is important (Table V). While none of 5 dogs fed the standard diet for only 4 weeks before renal injury was produced (subgroup 5) showed lesions, 9 of 11 dogs kept on the standard diet for 6 weeks or longer (subgroups 6, 10 and 12) showed lesions after comparable renal injury. Of the two failures in this group, 1 (dog 42-2) consumed the diet for only 6 weeks and the other (dog 40-83) lived only 3 days after bilateral nephrectomy. These

TABLE V
Influence of Length of Time on Standard Diet upon the Incidence of Arterial Lesions following Renal Injury

Time on standard diet	Subgroup numbers*	Number of animals	Number with arterial lesions	Per cent positive
Short (3 to 6 weeks)				
With plasma alteration	8, 11, 13, 9	14	13	92.9
Without plasma alteration	5	5	0	0
Long (6 weeks to 6 months)				
Without plasma alteration	6, 10, 12	11	9	81.0

*These subgroup numbers correspond with those in Table I of the following paper.¹⁰

studies are being continued in an attempt to define "optimum conditions" for the production of the arterial lesions.

Second, injections of citrated plasma obtained from healthy donor dogs accelerate or augment the production of the arterial lesions. This was the procedure used at the time these lesions were first observed;¹ and many experiments bear out this second point. Eleven of 12 dogs fed the standard diet for 3 to 5 weeks while they were receiving daily intravenous injections of homologous plasma (injections that averaged about one-third of the total plasma volume of the recipient and thus amounted in all to about five to six times the total plasma volume of the recipient, subgroups 8, 11 and 13) had typical arterial lesions when they died in "uremia" 5 to 17 days after the production of renal insufficiency. Two dogs fed the standard diet for 3 and 5 weeks respectively while they were made hypoproteinemic by plasmapheresis (subgroup 9) also had typical arterial lesions. This incidence is to be contrasted with that of no arterial lesions in 5 dogs, fed the standard diet alone for the same period (subgroup 5, Table V).

Third, with "optimum conditions" tentatively defined as repeated intravenous injections of donor's plasma for 3 to 5 weeks followed by the subcutaneous injection of 5.0 mg. of uranium nitrate per Kg., all 6 dogs fed the standard diet had lesions while none of 4 dogs fed a lean meat diet had lesions (Table VI).

The fourth point does not pertain directly to diet but may have a bearing on the problem as a whole. It grew out of control experiments on the data presented in the preceding paragraph, experiments designed to test the effect of the anticoagulant used in making the injections of donor's plasma.⁹ This anticoagulant was tri-sodium citrate, 1.25 cc. of a saturated aqueous solution (about 0.8 gm.) per 100 cc. of donor blood, and over 90 per cent of this was contained in the plasma that

TABLE VI
Influence of Diet under "Optimal" Conditions for the Production of Arterial Lesions*

Dietary group	Subgroup numbers†	Number of animals	Number with arterial lesions	Per cent positive
Standard diet	8	6	6	100.0
Lean meat diet	14, 15	4	0	0

* "Optimal" conditions are tentatively defined as repeated intravenous injections of donor's plasma for 3 to 5 weeks followed by 5.0 mg. of uranium nitrate per Kg.

† These subgroup numbers correspond with those in Table I of the following paper.¹⁰

was injected into the recipient. When saline plus citrate is injected intravenously instead of plasma plus citrate (the amount of citrate and total volume of fluid being kept constant) not only do the arterial lesions not develop but the dogs are protected against a lethal dose of uranium nitrate. Twelve of 13 (92 per cent) control dogs (without citrate) succumbed in 9 to 13 days to 5.0 mg. of uranium nitrate per Kg.; 13 of 14 (93 per cent) experimental dogs (with citrate) survived the same dose of heavy metal. This protective action was independent of diet. It is noteworthy that the "saline and citrate" did not prevent urinary, blood chemical and anatomical evidence of renal injury.⁹ Despite these definite evidences of renal injury, none of these experimental animals showed any evidence of healed arterial lesions when they were subsequently sacrificed. This fourth point can be stated very simply and directly: citrated homologous plasma augments the production of the arterial lesions; citrated saline protects against those following the injection of uranium nitrate. (Experiments to date have failed to show that citrate affords any protection against mercuric chloride injury¹¹ and it could not be expected to protect against bilateral nephrectomy.)

DISCUSSION

The chief point indicative of a dietary factor that has emerged from these studies is that with the same degree of renal injury about 90 per cent of the dogs fed the standard diet (22 of 25) have developed necrotizing arterial lesions, while only 5 per cent of 97 "control" dogs consuming an unknown amount of a variable kennel ration have developed similar lesions (Table IV). At present I have no definite evidence as to the nature of the dietary factor; *i.e.*, whether it is (1) a deficiency, (2) a toxic factor, or (3) an imbalance. Studies are under way at the present time in an attempt to isolate and define the dietary factor.

Necrotizing arterial lesions in dogs have been produced in other laboratories, but it is not certain that all of these lesions are identical. Goldblatt,⁷ using bilateral subtotal renal arterial constriction, has produced principally necrotizing arteriolitis. This localization—especially in the visceral arterioles—may be related to the hypertension that precedes and accompanies the arteriolar changes. The few scattered determinations of blood pressure which have been made in connection with these experiments have not yielded consistent results, but there is ample evidence that hypertension is not a necessary precursor to the lesions in the large elastic arteries. An occasional arteriolar change has been seen and illustrated in the myocardium,³ lungs, and submucosa of the stomach (never in the liver, pancreas, adrenals, or kidneys). By far the major damage to the arterial system seen in my dogs, however, has been in the large elastic arteries; in particular, the endocardium of the left auricle, the pulmonary artery, and the ascending limb of the arch of the aorta, including the mouth of the innominate artery.

Winternitz and his colleagues^{5, 6} have found "hemorrhagic" and "necrotizing" lesions in various organs and tissues following the production of renal insufficiency in dogs in a variety of ways, including bilateral nephrectomy and subtotal ureteral obstruction. Reference to the following paper¹⁰ indicates that I, too, have seen many lesions that could be described as "hemorrhagic" and/or "necrotizing." These lesions (stomatitis, gastroenteritis and pancreatic fat necrosis) are far less dependent on diet than are the arterial lesions which are referred to in this paper. If only lesions of the arterial tree are considered, it is not clear that the New Haven group has encountered the same lesions that I have. Most of the lesions which that group has illustrated have been in muscular arteries or in arterioles. In the few lesions in large elastic arteries which they illustrate, hemorrhage and necrosis without much leukocytic response characterize the lesions.

Polymorphonuclear neutrophils and "cells with distorted nuclei" dominate the lesions in my dogs, and, from the available descriptions, the lesions seem to be much more extensive than are those of Winternitz *et al.* Quantitative factors may be involved, for there are many factors in common between the arterial and other lesions (see following paper¹⁰) produced in this laboratory and those produced in the New Haven laboratory.

The aortas and pulmonary arteries of some of the "control" dogs fed the kennel diet and especially of the dogs fed the lean meat diet (subgroups 14 and 15) showed rather striking edema of the inner third of the media and the intima, and a few showed "bubble elevation" of the endothelial lining as if by edema; but hemorrhage, leukocytic reaction and necrosis were absent. Some of the lesions resemble those reported by Hueper¹² following the intravenous injections of macromolecular substances.

These differences in the arterial lesions produced by various procedures in various laboratories have been referred to in some detail, for none of these other laboratories has found it necessary to control the diet of its experimental animals. The work of the New Haven and Cleveland groups is stressed in particular, for there is one factor common to all of the recently published results⁵⁻⁷ from those laboratories as well as from this laboratory, namely, renal insufficiency. This seems to be *sine qua non* so far as the production of the necrotizing arterial lesions in question is concerned. If a dietary factor is involved, it is one that manifests itself only in the presence of renal insufficiency. The "standard diet" which my dogs have consumed is adequate, for dogs have been maintained on it in good clinical condition with no loss of weight for over a year. A borderline deficiency might be involved but this could probably be defined more accurately as depletion of a "reserve store" of some hypothetical factor. Quantitative factors are almost certainly involved, for under "optimum conditions for the production of the lesions," a shift to a lean meat diet completely alters the incidence of the lesions (Table VI). It is possible that the dogs on the lean meat diet stored some substance necessary for the maintenance of elastic arteries in sufficient amount to "buffer" them against the damaging effect of renal insufficiency.

Further speculation is unwarranted at this time. The immediate question is: Is a dietary factor involved? Our experience to date indicates that if normal adult dogs are fed the "standard diet" for 2 months or longer before being subjected to renal insufficiency by either mercuric chloride or uranium nitrate or by bilateral nephrectomy, 9 out of 10 (if they live 4 days or longer after the renal injury) will show

a necrotizing process in the large elastic arteries—in particular in one or more of these three sites: (1) endocardium of the left auricle, (2) pulmonary artery and (3) ascending limb of the arch of the aorta including the mouth of the innominate artery.

SUMMARY

Acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries) have been produced with regularity in dogs by controlling two factors: (1) renal insufficiency and (2) diet. Only the first of these factors has been confirmed in other laboratories.⁵⁻⁷ In this paper the preparation and feeding of the "standard diet" which has been used in this laboratory are described in detail. The data that indicate that a dietary factor is fundamentally associated with the lesions can be summarized as follows:

1. The strongest evidence for a dietary factor is that with the same degree of renal insufficiency, 88 per cent (22 of 25) of the dogs fed the "standard diet" have had typical arterial lesions while similar lesions have occurred in only 5.2 per cent of 97 "control dogs" fed the regular kennel diet. Arterial lesions in the "control dogs" are described and illustrated for the first time.

2. The period of feeding the "standard diet" before the production of renal insufficiency is important. Data obtained thus far indicate that a minimum of about 8 weeks is necessary, but this period can be greatly shortened by repeated intravenous injections of citrated plasma obtained from healthy donor dogs.

3. While citrate and homologous plasma augment the production of the lesions (shorten the period of "standard diet" feeding), saline citrate solution prevents the lesions and protects the dog against death after a dose of uranium nitrate that proved lethal to 12 of 13 controls.

4. The method by which renal insufficiency has been produced has not influenced the incidence of the arterial lesions except in so far as it has affected the survival period (the longer this period the more advanced the lesions).

5. Experiments are still in progress to determine "optimum conditions" for the production of these arterial lesions. Under "optimum conditions," tentatively defined as daily injections of homologous plasma six times per week for 3 to 5 weeks before the subcutaneous injection of 5.0 mg. of uranium nitrate per Kg., all 6 dogs fed the "standard diet" during this period showed typical arterial lesions while none of 4 dogs fed a diet of lean meat developed lesions.

All dogs in both the control group (kennel diet) and in the experi-

mental group (standard diet) that have developed arterial lesions have been subjected to renal injury, and the lesions have always corresponded, both grossly and histologically, to the interval between the production of renal insufficiency and death. Hence, if a dietary factor is involved in these arterial lesions, it is a dietary factor that manifests itself only in the presence of renal insufficiency. Before speculating on the nature of the dietary factor or on the manner in which renal insufficiency influences a dietary factor, it is first necessary to determine whether a dietary factor is involved. The data to date indicate that it is.

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DESCRIPTION OF PLATE

PLATE 122

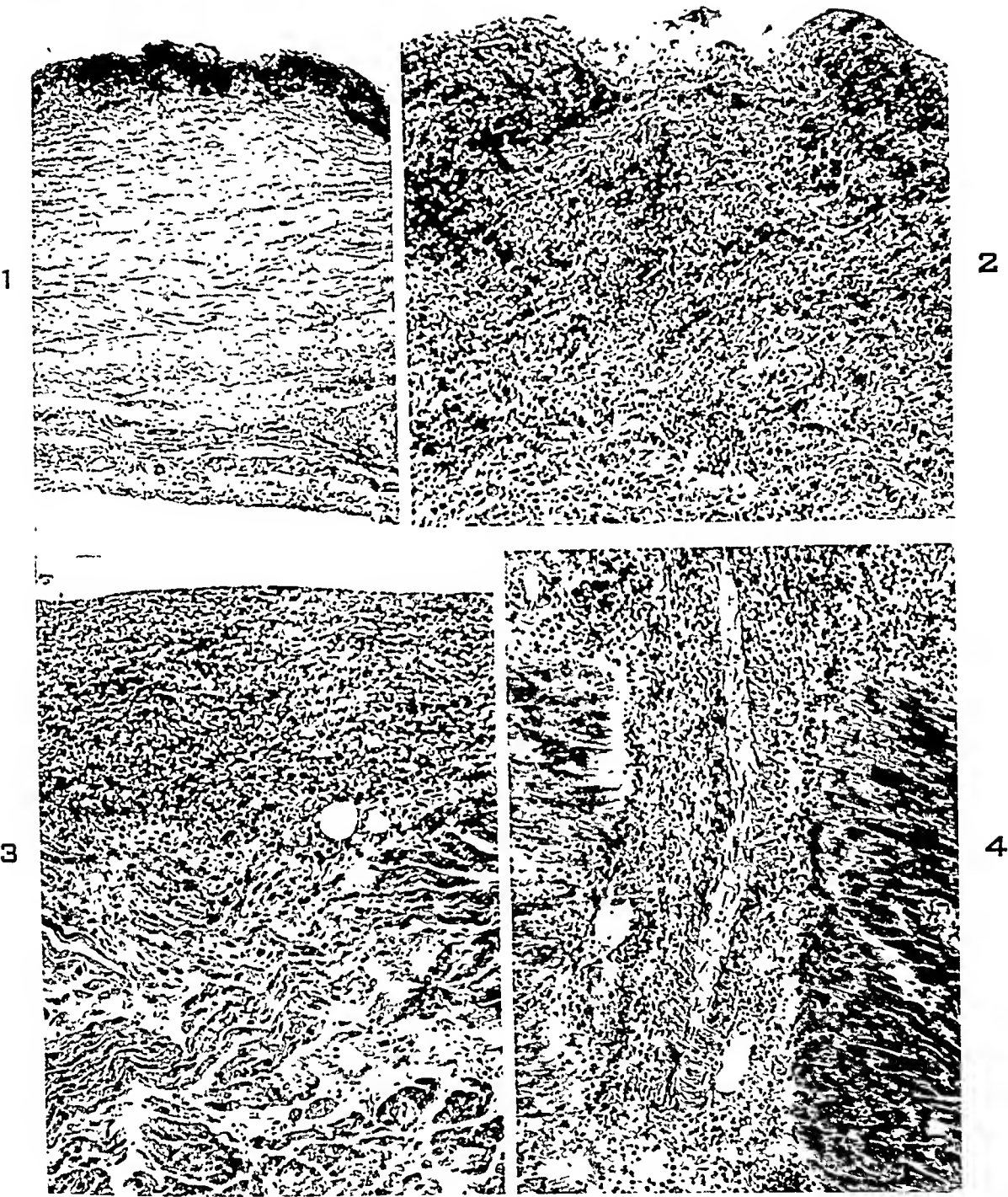
Necrotizing arterial lesions in "control" dogs. All sections are stained with hematoxylin and eosin.

FIG. 1. Dog K-42. Aorta. Acute necrotizing aortitis of inner portion of wall. Edema of remainder of media. $\times 50$.

FIG. 2. Dog 40-72. Pulmonary artery. Acute necrotizing arteritis. $\times 140$.

FIG. 3. Dog 40-88. Acute necrotizing left auriculitis. $\times 140$.

FIG. 4. Dog 40-72. Acute periarteritis of myocardial arteriole. $\times 140$.



Holman Experimental Necrotizing Arteritis. V

NECROTIZING ARTERITIS IN DOGS RELATED TO DIET AND RENAL INSUFFICIENCY

VI. ASSOCIATED LESIONS: STOMATITIS, GASTROENTERITIS AND PANCREATIC FAT NECROSIS*

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In the preceding paper¹ and in previous publications²⁻⁵ some of the anatomical changes that follow the production of renal insufficiency in dogs have been presented. In reports thus far, attention has been focused on the changes in the arterial system, especially in the large elastic arteries such as the aorta, endocardium of the left auricle, pulmonary and coronary arteries. In essence these changes amount to an acute necrotizing arteritis not unlike that encountered in human cases of rheumatic arteritis and periarteritis nodosa, but no claim of identity has been made. The data to date have supported the belief that these changes are related to a dietary factor that manifests itself only in the presence of renal insufficiency.

Lesions in three other anatomical sites have occurred in these dogs with sufficient frequency to relate them to at least one part of the experimental procedure (renal insufficiency). These sites and the corresponding lesions are: (1) mouth—stomatitis; (2) gastrointestinal tract—gastritis, enteritis and colitis; and (3) peripancreatic fat—pancreatic fat necrosis. It is the purpose of this paper to describe these lesions and to try to interpret them in the light of the various experimental procedures. The experimental data afford evidence (1) that “mercurial” stomatitis and gastroenteritis are not due to mercury as such but to the renal insufficiency caused by the heavy metal, and (2) that classical examples of acute inflammation can be caused by an “internal irritant.”

METHODS

The methods are the same as those described in the preceding paper.¹

EXPERIMENTAL OBSERVATIONS

The data in this paper are restricted to the 57 dogs on which complete necropsies were performed. The pertinent data on these dogs are given in Table 1. The same method of grading lesions has been used throughout: 3 plus = gross lesion over 1 cm. in maximum diameter; 2 plus = gross lesion less than 1 cm. in maximum diameter; 1 plus =

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TABLE I
Summary of Experimental Data

Dog no.*	Sex	Estimated age (yrs.)	Body weight at time of renal injury (Kg.)	Diet†	Period on diet before renal injury (weeks)	Renal injury produced by‡	Plasma alteration§	Survival interval (days)	Maximum N. P. N. (mg./100 cc.)	Lesions in				Subgroup no.
										Art-eries¶	Mouth	Gastro-intestinal tract	Peripan-creatic fat	
41-94	F	1	3.9	Ken.		U 5.0		6	446	—	+	+	—	1
42-27	F	4	4.3	Ken.		U 5.0		6	552	—	+	—	—	
41-92	F	5	6.3	Ken.		U 5.0		9	388	—	+	—	—	
41-93	F	1	4.4	Ken.		U 5.0		11	610	—	+	+	+	
41-91	M	1	8.3	Ken.		U 5.0		28 ^s	160	—	—	—	—	
40-78	F	2	6.2	Ken.		U 5.0	Citrate	3 ^s	86	—	—	—	—	2
40-75	F	5	6.9	Ken.		U 5.0	Citrate	6 ^s	288	—	—	—	—	
40-70	F	1	7.0	Ken.		U 5.0	Citrate	9 ^s	495	—	+	—	—	
40-58	F	2	7.2	Ken.		U 5.0	Citrate	12 ^s	642	—	+	—	—	
40-65 ^p	F	4	20.3	Ken.		Hg 2.0		7	403	—	+	—	—	3
41-97	F	2	14.8	Ken.		Hg 2.0		9	531	—	—	—	—	
40-66 ^p	F	4	20.1	Ken.		Hg 2.0		10 ^s	112	—	—	—	—	
40-72 ^p	F	4	24.8	Ken.		Hg 2.0		10	523	+	+	+	+	
39-35 ^p	M	6	26.0	Ken.		Hg 2.5		3	194	+	+	+	+	
39-36 ^p	M	5	23.0	Ken.		Hg 2.5		3	178	+	+	+	+	
39-47 ^p	M	4	18.4	Ken.		Hg 2.5		3	311	—	+	+	+	
39-37 ^p	M	6	22.0	Ken.		Hg 2.5		4	244	—	+	—	—	
39-39 ^p	M	6	19.6	Ken.		Hg 2.5		4	207	—	+	—	—	
40-88	M	1	17.5	Ken.		Hg 3.0	Citrate	4	330	+	+	+	+	4
41-96	F	1	12.0	Ken.		Hg 3.0	Citrate	11	692	—	+	—	—	
40-53	F	1	7.4	Stan.	4	U 5.0		10	531	—	+	—	—	5
40-54	F	1	9.4	Stan.	4	U 5.0		12	448	—	+	+	+	
40-51	M	1	7.8	Stan.	4	U 5.0		13	337	—	+	+	+	
40-57	M	1	9.2	Stan.	4	U 5.0		21	457	—	+	+	+	
39-33	F	2	8.2	Stan.	4	U 5.0		122 ^s	151	—	—	—	—	
42-7	M	1	4.0	Stan.	10	U 5.0		10	348	+	+	+	+	6
42-8	F	1	6.8	Stan.	10	U 5.0		10	442	+	+	—	—	
42-5	F	2	6.8	Stan.	12	U 5.0		16	363	+	+	—	—	
40-68	F	5	3.9	Stan.	12	U 5.0		35 ^s	162	+	+	—	—	
40-79	F	1	4.5	Stan.	16	U 5.0		7	544	+	+	+	+	

39-46 39-48 39-47	F M M	3 2 1	8.1 9.5 9.0	Stan. Stan. Stan.	4 4 4	U 5.0 U 5.0 U 5.0	Citrate Citrate Citrate	160 ^s 244 ^s 900 ^s	116 49 75	— — —	— — —	7
39-34 39-40 39-45 39-43 40-50 39-28	M M F F F F	2 1 2 3 1 4	4.4 6.5 7.3 4.7 4.8 5.1	Stan. Stan. Stan. Stan. Stan. Stan.	4 3 4 5 4 5	U 5.0 U 5.0 U 5.0 U 5.0 U 5.0 U 5.0	Pl. inj. Pl. inj. Pl. inj. Pl. inj. Pl. inj. Pl. inj.	8 11 14 15 17 345 ^s	297 582 617 438 573 92	— + + + + —	— + + + + —	8
38-24 39-49	F M	3 2	4.5 4.8	Stan. Stan.	6 3	U 3.0 U 5.0	Hypo. Hypo.	15 15	386 391	— —	— —	9
42-3 42-4 42-2	F F F	3 1 2	5.3 3.5 5.7	Stan. Stan. Stan.	6 6 6	Hg 3.0 Hg 3.0 Hg 3.0		4 5 6	289 374 436	— + —	— + —	10
40-80 40-63 41-89	F F M	2 2 2	5.8 9.2 8.0	Stan. Stan. Stan.	4 4 4	Hg 3.0 Hg 3.0 Hg 3.0	Pl. inj. Pl. inj. Pl. inj.	6 7 12 ^s	466 394 181	— + —	— + —	11
40-83 40-85 40-84	F F M	1 1 3	6.3 7.1 7.4	Stan. Stan. Stan.	23 23 19	B. N. B. N. B. N.		3 4 5	348 308 244	— + +	— + —	12
40-52 42-1 40-77	F F F	2 1 1	7.3 5.6 5.2	Stan. Stan. Stan.	4 6 4	B. N. B. N. B. N.	Pl. inj. Pl. inj. Pl. inj.	5 6 7	710 538 295	— + +	— + +	13
40-60 40-59 40-64	M F F	1 2 3	6.3 5.8 4.9	L. M. L. M. L. M.	4 4 4	U 5.0 U 5.0 U 5.0	Pl. inj. Pl. inj. Pl. inj.	10 13 180 ^s	638 782 239	— — —	— — —	14
40-67	F	1	4.3	L. M.	4	Hg 2.5	Pl. inj.	81 ^s	59	—	—	15

* Superscript D in subgroup 3 indicates that dog was used as a donor for a year or longer before being subjected to renal insufficiency.

† Ken. = kennel diet; Stan. = "standard diet"; L.M. = lean meat diet.

‡ U 5.0 = uranium nitrate, 5.0 mg./Kg. subcutaneously; Hg 3.0 = mercuric chloride, 3.0 mg./Kg. intravenously; B.N. = bilateral nephrectomy.

§ Citrate = intravenous injections of sodium citrate; Pl. inj. = intravenous injections of citrated plasma obtained from "donor" dogs; Hypo. = hypoproteinemia by plasmapheresis.

|| Superscript S indicates that the dog was sacrificed on this day after renal injury was produced. Numbers without "S" indicate period after renal injury at which dog died.

¶ Superscript H = healing or healed; superscript A = aneurysm.

lesion discovered in microscopical sections. The largest lesion encountered served as the basis for this grading. For purposes of completeness and comparison, the arterial lesions discussed in the preceding paper¹ have been listed in this table.

Stomatitis

More data are available on the mouth lesions, for they can be followed from day to day. The method by which renal insufficiency was produced has influenced the time of appearance of the lesions. When mercuric chloride and bilateral nephrectomy were used for this purpose, early lesions have been observed as soon as 36 hours after the renal injury and typical gross lesions have been found when the dog died in the next 36 hours, *i. e.*, 3 days after the production of the renal injury (*cf.* dogs 39-35 and 40-83). When uranium nitrate was used as the nephropathic agent, mouth lesions did not appear before the 4th day and sometimes did not appear until 2 or 3 days before the animal died on the 14th to 17th day after the uranium nitrate was injected. Healing or healed lesions have been observed in 2 dogs that survived the renal injury.

In the majority of cases the mouth lesions have occurred in three locations. The first lesions are usually found on the mucous membrane of the upper lip opposite the alveolar ridge of the premolar teeth on either side. Careful daily examination of this area reveals the first change as an area of slightly dilated capillaries. The mucous membrane over the area is dry and within a short time becomes delicately "sanded" (as if by fibrin). Within 24 hours after these changes definite necrosis with the appearance of patches of grayish yellow exudate are evident. These patches frequently have a thin reddish margin of dilated capillaries, and small hemorrhages have occurred in some of the patches. This same series of events occurs at the other two sites in the oral cavity, namely, the mucous membrane of the cheeks about 1 cm. posterior to the angles of the mouth and on the under surface of the distal half of the tongue near the lateral margins. In advanced cases the necrotic patches on the cheeks have extended forward to join the posterior spread of the lesions of the upper lip, but even in these cases the mucous membrane of the lower lip escapes. The average lesion found at necropsy measures about 12 mm. in diameter (Fig. 1).

By the time the lesions make their appearance the breath is usually fetid, but the odor due to the lesions is oftentimes overshadowed by the odor of "uremia." In these terminal stages there is much drooling of mucoid saliva, which frequently is blood-tinged.

Microscopical examination of the lesions confirms the gross impression that the earliest changes occur beneath intact epithelium. Congestion and edema with margination and emigration of polymorphonuclear neutrophilic leukocytes and outpouring of fibrin have all been observed beneath intact squamous epithelium (Figs. 2 and 4). The majority of the sections show advanced changes with massive polymorphonuclear infiltration, obvious necrosis, and marked bacterial growth on the surface. Even in these advanced cases it is surprising how frequently remnants of the squamous epithelium are recognizable (Figs. 3 and 5). In other words, even the advanced lesions give evidence that ulceration with subsequent invasion of the tissues by mouth organisms was not the first change. Bacterial stains have shown the usual mouth organisms, including spirochetes and fusiform bacilli in some of the sections. In the healing and healed lesions, monocytic removal of debris, granulation tissue becoming progressively less vascular and more fibrous with time, and re-epithelization of the surface have all been observed. The changes are insignificant in the oldest healed lesion.

The incidence of these mouth lesions is much the same in the various groups of experimental animals and is much more independent of diet than are the arterial lesions described in the preceding paper.¹ Their occurrence by method of production of renal insufficiency, by diet and by various "plasma alterations" is indicated in Table II. Forty-two of the 57 dogs (74 per cent) had mouth lesions. All 6 dogs subjected to bilateral nephrectomy, regardless of "plasma alteration," had the lesions, thus excluding the possibility that the lesions are due to heavy metal. Only one group of animals is of interest for the absence of lesions. None of the 3 dogs that received intravenous injections of sodium citrate before being injected with uranium nitrate (39-46, 39-47 and 39-48, subgroup 7) developed mouth lesions. Other published results⁶ confirm this finding that sodium citrate protects dogs against uranium nitrate injury. The factor responsible for these mouth lesions remains obscure just as it does in human cases of mercurial stomatitis.

Gastroenteritis

The lesions in the gastrointestinal tract have been divided into gastric (present in 18 of the 57 dogs, or 32 per cent), small intestinal (present in 11 of the 57 dogs, or 19 per cent), and large intestinal (present in 6 of the 57 dogs, or 11 per cent). In all three locations the lesions are essentially similar and are characterized grossly by hemorrhage. The size and distribution of the hemorrhagic lesions in the gastrointestinal tract have varied markedly. In the stomach they

TABLE II
Influence of Method of Production of Renal Insufficiency, Diet and "Plasma Alteration" on the Incidence of Lesions

Renal insufficiency produced by	Diet	"Plasma alteration"	No. of dogs	Number with lesions in						Subgroup no.*	
				Mouth	Gastrointestinal tract				Peripan- creatic fat		Arteries
					Stomach	Small intestine	Colon	Total			
Uranium nitrate	Kennel	None	5	4			2		2	(1) (2)	
		Citrate injections	4	2							
	"Standard"	None (4 wks. of diet)	5	3	1	1	1	3	1	(5) (6)	
		None (10 wks. of diet)	5	4	3	1	1	3	1	(6) (7)	
		Citrate injections	3							(7) (8)	
		Plasma injections	6	4	4	2	1	5	6	(8) (9)	
		Hypoproteinemia	2	2	2			2	2		
	Lean meat	Plasma injections	3	2		1	1	1		(14)	
	Total		33	21 (64)	10 (30)	7 (21)	4 (12)	16 (48)	3 (9)	13 (39)	
	Percentage with lesions										
Mercuric chloride	Kennel	None	9	7	1		2	1	2	(3) (4)	
		Citrate-injections	2	2				1	1		
	"Standard"	None (6 wks. of diet)	3	3	1			1	1	2	(10)
		Plasma injections	3	3	2	1	1	3	1	2	(11)
	Lean meat	Plasma injections	1							(15)	
	Total		18	15 (83)	4 (22)	3 (17)	2 (11)	7 (39)	5 (28)	6 (33)	
	Percentage with lesions										
	Bilateral nephrectomy	"Standard"	None (20 wks. of diet)	3	3	1	1	2	1	2	(12)
			Plasma injections	3	3	3			3	2	(13)
		Total		6	6 (100)	4 (67)	1 (17)		5 (83)	3 (50)	5 (83)
	Percentage with lesions										
	Total for all dogs		57	42 (74)	18 (32)	11 (19)	6 (11)	28 (49)	11 (19)	24 (42)	
	Percentage with lesions										

* These subgroup numbers correspond to those in Table I.

have varied from a few millimeters in diameter to massive lesions involving practically the entire fundic portion. The gastric lesions were confined to the acid-secreting portion. In the most advanced case (dog 40-63, subgroup 11) there was a heavy hemorrhagic fibrinous exudate which formed a membrane that covered the fundus. This measured 8 mm. in its thickest portion. Histologically the early lesions were characterized by hemorrhage in the mucosal stroma and edema in the submucosa. In both of these locations there were slight deposits of fibrin and early infiltration with polymorphonuclear neutrophils. As the lesions became more advanced, fibrin and polymorphonuclears with varying degrees of necrosis occupied a more conspicuous part of the picture, but even in the most advanced lesions hemorrhage and edema were the predominant features. In 2 of the dogs with gastric lesions there were acute necrotizing lesions in the arterioles in the submucosa (Fig. 6), but there was no obvious cause-and-effect relationship between these two concurrent lesions.

In the small intestine the lesions consisted principally of scattered clusters of small hemorrhages. A typical cluster contained ten to fifteen more or less rounded hemorrhages measuring about 3 mm. in diameter, the whole confined to a segment of the bowel measuring about 6 cm. in length. These clusters of petechial hemorrhages occurred in every portion of the small intestine but were found most frequently in the duodenum and upper jejunum. When there was parasitic infestation (*e.g.*, hookworms) in this portion of the bowel the lesions bore no relationship to the parasites; and other dogs with heavier parasitic infestation had no intestinal lesions in the sense used in this paper. Histologically these lesions were similar to those in the stomach but were not as massive. One dog had an intussusception of its ileum.

In the colon the lesions tended to be most prominent in the cecum and in the ascending portion. Here hemorrhagic streaks along the crests of the longitudinal folds characterized the picture. In two of the dogs, patchy areas of shallow ulceration covered by a delicate membranous exudate were present about these hemorrhagic streaks. Microscopically the lesions were similar in character to those in the stomach and small intestine.

In all three segments of the gastrointestinal tract many more lesions were diagnosed grossly than were confirmed histologically. Only those confirmed by microscopical study have been included in this series. It is possible that a single microscopical section failed to show the lesion and that the incidence of lesions listed in Table II is too low; but in those cases with suspected gross lesions, microscopical study

revealed sufficient congestion to account for the gross appearance. Had these dogs lived longer, the congestion might have progressed to hemorrhage, exudation and necrosis. This same argument, however, could be advanced for every capillary in the body, hence it was thought best to include only those lesions confirmed by histological study.

Clinically these gastrointestinal lesions were usually associated with bloody diarrhea during the last day or two of life. This fact and the gross and histological appearance of the lesions, specifically the absence of sloughing and deep ulceration, make it probable that these lesions occurred within 3 days or less prior to death. Analysis of the data in Table II indicates that these lesions were not necessarily related to diet or to the method by which renal insufficiency was produced. The highest percentage of incidence was in the group subjected to bilateral nephrectomy. This excludes heavy metal as the causative agent, but the number of dogs is too small to attach any significance to the increased incidence of lesions in this group. As in the oral cavity, the lesions apparently started beneath intact epithelium and only after superficial ulceration did intestinal organisms add their enzymatic action to the inflammatory response. The factor responsible for these gastrointestinal lesions remains obscure just as it does for those in human cases of mercurial gastroenteritis.

Pancreatic Fat Necrosis

The third lesion that occurred with some degree of regularity in this series of dogs subjected to renal insufficiency is pancreatic fat necrosis. In no instance were these grayish white areas of necrosis particularly conspicuous, and it is questionable whether they played any significant part in the summation of chemical reactions leading to death. It seems more likely that they represent another anatomical evidence of a more fundamental disturbance associated with renal insufficiency. They were usually located in the peripancreatic fat; the single, most frequent site was the head of the dorsal pancreas near the duodenum. Occasionally they have been found in the periadrenal fat and in the areolar tissue about the ovaries, and in one instance in the mesentery. No lesions as large as 1 cm. in maximum diameter were encountered. Most of the lesions measured 2 to 3 mm. in maximum diameter and consisted of a small gray or grayish white dot. Sometimes the borders of these areas were reddish, but in no instance was any definite gross hemorrhage observed in association with these lesions. Microscopically these lesions were typical pancreatic fat necroses, usually with massive polymorphonuclear leukocytic reaction in, and especially about, the central area of fat

necrosis. The slurred bluish gray material (seen in sections stained with hematoxylin and eosin) that filled the space previously occupied by the fat cells was conspicuous in the central portion. In a few cases, sheaves of fatty acid crystals have been seen in this material. One of the lesions showed evidences of healing.

There were no clinical symptoms referable to these pancreatic fat necroses. They were not related to diet or to the method by which renal insufficiency was produced. Again their more frequent occurrence in the dogs subjected to bilateral nephrectomy excludes heavy metal from any etiological rôle. The factor responsible for these lesions is also obscure.

Other Lesions

In addition to the three lesions described, the following lesions have occurred in one or more of the dogs detailed in Table I:

- (a) Hemorrhages in epicardium, usually most marked along the atrioventricular sulcus.
- (b) Hemorrhages in endocardium. These have been most conspicuous in the left ventricle at the tips of the papillary muscles and in the septum below the aortic valve. Rarely has one of these hemorrhages been associated with necrosis of the myocardium.
- (c) Acute mitral valvulitis.
- (d) Edema of the tricuspid valve.
- (e) Hemorrhages in thymus.
- (f) Atrophy of lymphoid tissue.

These lesions seem to be related to the experimental procedures but their incidence has not been sufficiently great to warrant statistical analysis. Incidental findings, such as parasitic infections and infestations and terminal pneumonia, have not been included.

DISCUSSION

The lesions described in this paper (stomatitis, gastroenteritis and pancreatic fat necrosis) appear to be independent of diet. This contrasts sharply with the arterial lesions (described in the preceding paper ¹) in which diet seems to play a definite rôle. The lesions in the gastrointestinal tract, also some of the "other lesions" listed, are similar to those described by Winternitz and his co-workers.^{7, 8} These are discussed in the preceding paper.¹ No mention was made by these workers of mouth lesions or lesions in the peripancreatic fat.

Renal insufficiency is the only factor common to all of the lesions (including arterial lesions). The method by which renal insufficiency

is produced is unimportant. The fact that all of the lesions occurred as frequently, or more frequently, in dogs subjected to bilateral nephrectomy eliminates heavy metal *per se* from a pathogenic rôle. If these findings are confirmed for man, and I know of no contradictory evidence, "mercurial" stomatitis, gastritis and colitis are probably due not to mercury as such but to the renal insufficiency produced by the heavy metal.

We have no evidence as to the nature of the factor associated with renal insufficiency that is responsible for these lesions, *i.e.*, whether there is a deficiency of a necessary factor, a "toxic" factor, or an "imbalance."

The rate of development of renal insufficiency seems to be important and probably explains why all human cases of "uremia" do not develop these lesions. Whatever the responsible factor happens to be, it must occur at such speed that local breakdown products (degenerated collagen?) accumulate in sufficient concentration to disturb local equilibria and lead to hemorrhage and necrosis. Do the sites of predilection for the lesions—three in the mouth (upper lips, cheeks and under surface of the distal half of the tongue near the lateral margins), the stomach, the upper portion of the small intestine and the first portion of the colon, three in the arterial system (pulmonary artery, endocardium of the left auricle and ascending portion of the arch of the aorta including the mouth of the innominate artery) and the peripancreatic fat—have any anatomical or physiological peculiarities that would help explain these lesions? This question cannot be answered at this time, but the predictable frequency with which these lesions occur at definite anatomical sites, especially the three localized areas in the mouth and the three areas in the arterial system, following a general metabolic disturbance such as renal insufficiency (or renal insufficiency plus diet in the case of the arterial lesions) suggests that competitive affinities for a necessary factor or increased susceptibilities to a toxic factor may be in operation. I am inclined to favor the former view, namely, competitive affinities for a necessary factor, possibly because of the influence of diet on the incidence of arterial lesions, but lack of proof must be admitted.

Another feature of these lesions is noteworthy but I hesitate to do more than mention it for fear of getting sidetracked in a philosophical discussion. These lesions fill all the criteria for acute inflammation, yet as far as I can determine they are due only to alterations in kidney function (and diet in the case of the arterial lesions). There seems to be little or no doubt that the lesions start beneath intact epithelium in the case of the mouth and gastrointestinal lesions, be-

neath intact mesothelium in the case of the pancreatic fat necrosis and beneath intact endothelium in the case of the arterial lesions. Only when necrosis with sloughing and ulceration has opened a portal of entry to mouth and intestinal organisms do bacteria play a part in the inflammatory reaction. In the arterial lesions and the areas of pancreatic fat necrosis all attempts to demonstrate organisms by cultural methods or by bacterial stains have been negative. All of the control data² and the fact that the development of the lesions has always corresponded to the interval between the production of renal insufficiency and death militate against an infectious agent. The evidence that the lesions are due to an "internal irritant" seems to be valid. Other probable examples of the same phenomenon (inflammatory lesions due to an "internal irritant") could be cited, both in animal experiments⁹⁻¹¹ and in human pathology (periarteritis nodosa, muscular dystrophy), but it is not the purpose of this paper to discuss the mechanisms of inflammation.

SUMMARY

1. In a group of 57 dogs subjected to renal insufficiency by one of three methods (uranium nitrate in 33, mercuric chloride in 18, and bilateral nephrectomy in 6) the following lesions, in addition to the arterial lesions described in the preceding paper,¹ were found in the stated percentage of cases:

- (a) Acute necrotizing stomatitis in 42 (74 per cent)
- (b) Acute hemorrhagic gastroenteritis in 28 (49 per cent)
- (c) Acute pancreatic fat necrosis in 11 (19 per cent)

2. The occurrence of all three lesions in dogs subjected to bilateral nephrectomy seems to eliminate heavy metal *per se* from any pathogenic rôle in the development of these lesions.

3. The "irritant" responsible for the inflammatory reaction in all of these lesions appears to be an "internal" one related to the disturbed metabolism associated with renal insufficiency.

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DESCRIPTION OF PLATES

PLATE 123

Acute necrotizing stomatitis. All sections were stained with hematoxylin and eosin.

FIG. 1. Dog 42-5. Typical upper lip lesions.

FIG. 2. Dog 40-84. Upper lip. Intense leukocytic reaction beneath remnants of squamous epithelium. $\times 100$.

FIG. 3. Dog 41-93. Tongue. Extensive necrosis and leukocytic infiltration without excavating ulceration. $\times 84$.

FIG. 4. Dog 39-36. Tongue. Congestion, edema, hemorrhage, polynuclear infiltration and necrosis with relatively slight changes in the surface epithelium. $\times 135$.

FIG. 5. Dog 39-49. Tongue. Extensive leukocytic infiltration and necrosis with remnants of the surface epithelium still recognizable. $\times 140$.

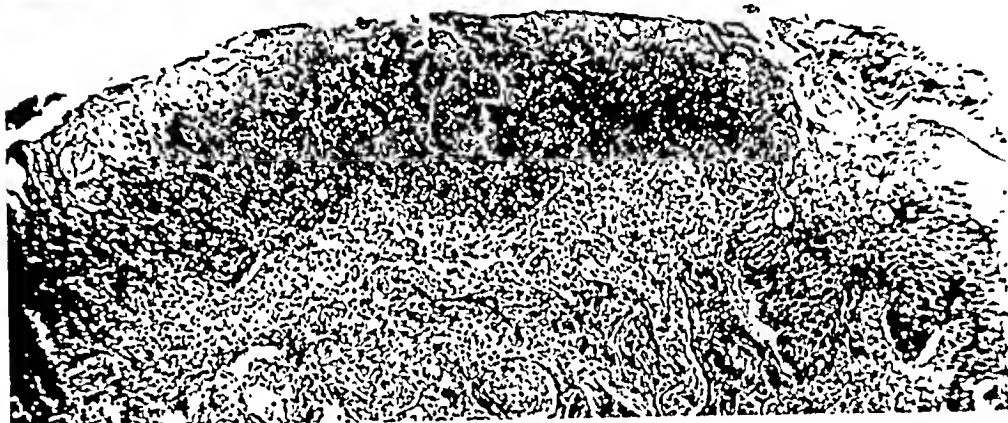
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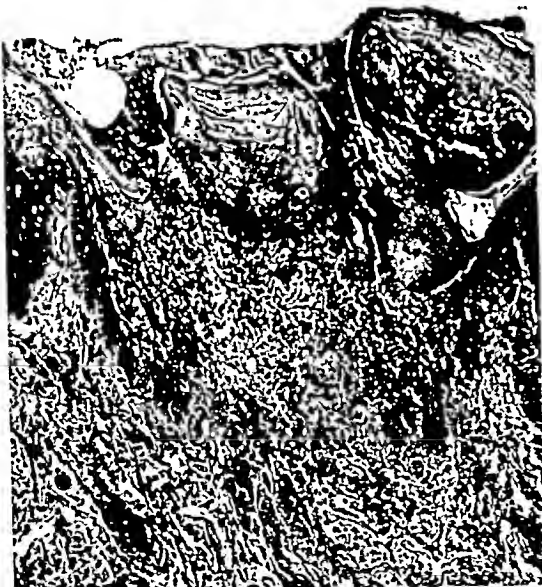
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Holman

Experimental Necrotizing Arteritis. VI

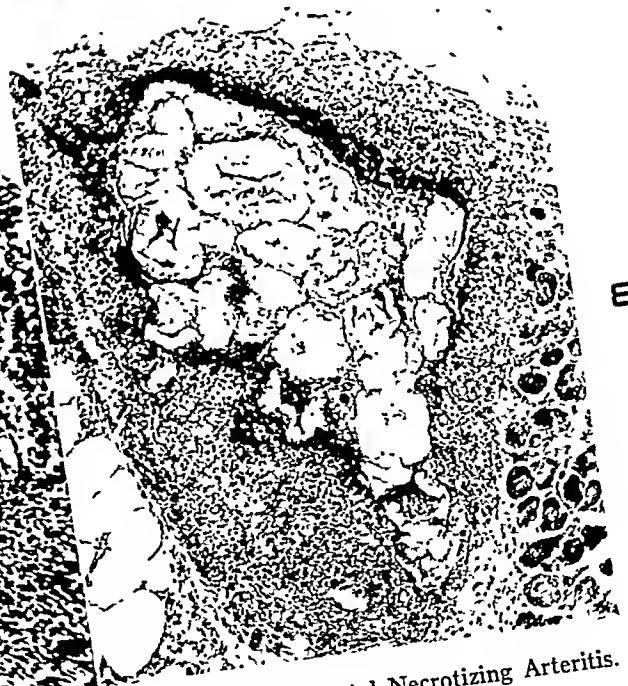
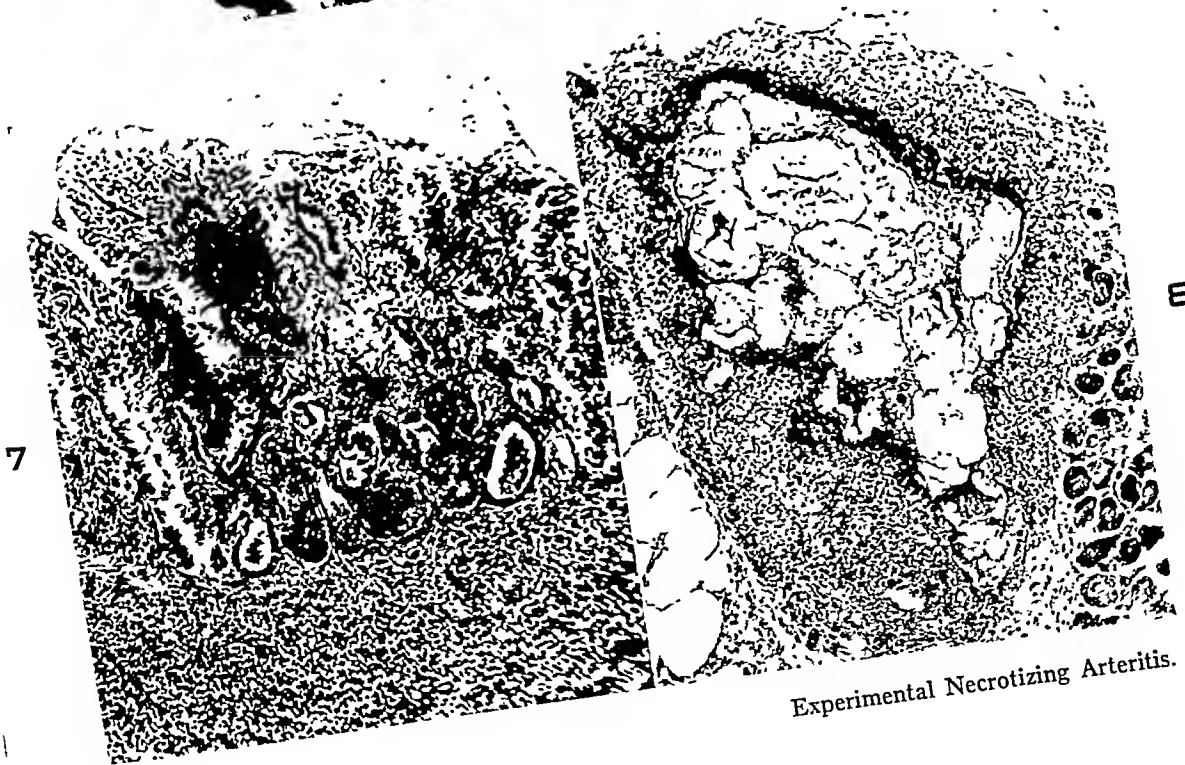
PLATE 124

All sections were stained with hematoxylin and eosin.

FIG. 6. Dog 42-8. Acute periarteritis of arteriole in submucosa of stomach. $\times 580$.

FIG. 7. Dog 41-94. Hemorrhage and early leukocytic infiltration in mucosal stroma of ileum. $\times 100$.

FIG. 8. Dog 40-63. Acute pancreatic fat necrosis of peripancreatic fat. $\times 100$.



Experimental Necrotizing Arteritis. VI

BACTERIOLOGICAL OBSERVATIONS ON EXPERIMENTAL BRUCELLOSIS IN DOGS AND SWINE*

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From the time of the earliest investigation of brucellosis it has been recognized that the host-parasite relationship in this disease presents a peculiar and puzzling problem. As the human disease has become better known the problems have multiplied, and it has become desirable to attempt a solution of them through investigations of the disease in the experimental animal. Furthermore, it has been suggested by recently reported studies¹⁻³ that brucella may be recovered from a considerable proportion of the cases of Hodgkin's disease, and, at the same time, it has been observed that there are striking similarities between human brucellosis and Hodgkin's disease.^{1, 4} The latter observations emphasize more than ever the need for more precise information concerning the relation between brucella and its hosts since only through such information can one expect to form an intelligent judgment of the significance of the data in those instances of a co-existence of brucellosis and Hodgkin's disease. In the latter connection, and also in studying the usual form of human brucellosis, one is faced with certain serious difficulties; the more immediately important of these may be stated briefly as follows: (1) As yet there appears to be no absolutely reliable method for the isolation of brucella from the tissues. (2) In cases of brucellosis proved by the recovery of brucella from the blood, the bacteremia appears to be intermittent, the time between its repeated occurrence being extremely variable; this condition is impossible to understand until the reservoir of the organisms responsible for these recurrences of the bacteremia is known with certainty. (3) The precise relation of the cells of the natural host to the organisms that may be obtained from the tissues by culture is not fully understood. (4) The factors which determine the prolonged course and the recurrence of attacks in chronic brucellosis have not been determined. (5) The influence of the host tissues upon the pathogenicity of organisms which remain in the tissues for long periods is only approximately known. (6) The specific mechanisms through which brucella provokes the different defense mechanisms of the host are little understood.

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During the past 2 years we have carried out a series of studies of experimentally induced brucellosis in a variety of animals in an attempt to solve some of these problems. A preliminary report of the general pathological findings in these studies as they relate to the dog and the hog may be found in the Proceedings of the American Association of Pathologists and Bacteriologists.⁵ The report which is to follow covers the bacteriological and immunological observations that were made on this same group of animals. In our experiments on the hog, a natural host, and the dog, an unusual host, our chief object was to learn, first, how long the organisms remain in the tissue when they are no longer demonstrable in the peripheral blood, and, second, under such circumstances, in what tissues they may be found most frequently.

Instances of proved spontaneous brucellosis in the dog are few. Kennedy and Eyre⁶ obtained four positive agglutination tests for *Brucella melitensis* in 162 dogs tested in Malta. The bacterium was isolated from the tissues of one of these dogs at autopsy. Plantz and Huddleson⁷ reported the isolation of *Brucella suis* in pure culture from a testicular abscess in a 3½-year-old dog with a blood serum agglutinating titer of 1:500 by rapid test. Van der Hoeden⁸ isolated brucella from the blood of a dog with high agglutinating titer by inoculating a guinea-pig with the blood. Two other dogs with high blood serum titers yielded negative blood cultures. The organism could not be recovered from the liver or the kidney of any of the 3 animals either by direct culture or by guinea-pig inoculation. Reports of the occurrence in dogs of specific agglutinins for brucella have been made by various investigators. This is reviewed by van der Hoeden,⁸ Caliri⁹ and Feldman, Mann and Olson.¹⁰ In van der Hoeden's⁸ review are reported the results of his own experiments in which 11 dogs were infected *per os* with various strains of brucella. Agglutinating and complement-fixing antibodies were regularly found in these animals from 6 days to 3 months following introduction of the organisms. At autopsy, brucella was recovered from 7 animals, the spleen, liver, bone marrow and mesenteric lymph nodes yielding the higher percentages of positive cultures (54.5 to 57.1 per cent). The organism was recovered less frequently from the blood and rarely from the kidneys, bile, or testis. One dog receiving heat-killed brucella *per os* failed to develop demonstrable serum antibodies during the 35 days it was observed. In a later investigation, van der Hoeden¹¹ reported a group of experiments in dogs in which infection had been produced *per os*, percutaneously by way of the conjunctiva, and by contact with dogs previously infected *per os*. These animals were killed from 1 to

135 days following infection. *Brucella* was recovered more frequently from the spleen, liver, bone marrow and regional lymph nodes (47.6 to 65.2 per cent), less frequently from the blood (22 per cent) and rarely from kidneys and testis. Van der Hoeden called attention to the most frequent involvement of parts of the reticulo-endothelial system. Feldman, Bollman and Olson¹² recovered brucella at autopsy from 2 of 5 dogs infected intravenously. One of these animals had positive blood and urine cultures when sacrificed on the 39th day. The other did not yield positive cultures during life; at autopsy, 185 days after the inoculation, the organism was recovered from a mesenteric lymph node. Of 6 dogs infected *per os*, 3 yielded positive blood cultures on the 14th day, but no positive cultures were obtained at autopsy. On the basis of these observations Feldman, Bollman and Olson expressed the opinion that spontaneous brucellosis in dogs is rare, since this animal apparently possesses a highly efficient mechanism for protection against *Brucella abortus*.

Both spontaneous and experimental infections with brucella in swine have received considerable attention in recent years. Good and Smith¹³ isolated brucella from the fetuses of an aborting cow and inoculated this organism into two pregnant sows intravenously and *per os*. The sows aborted 17 and 19 days, respectively, after infection, and from the fetuses of each brucella was recovered. Weeter¹⁴ reviewed the literature dealing with the early investigations of spontaneous infection of swine and contributed further observations on spontaneous and experimental infection of swine with brucella. In Weeter's observations on spontaneous infection, the organism was isolated three times from 259 nongravid uteri of swine and once from 289 gravid uteri. His attempts to infect adult swine *per os* resulted in the production of agglutinating antibodies in the serum of the animals, but the initial infection and subsequently ingested organisms soon were eliminated completely as indicated by failure to recover the organisms. Young animals showed a high resistance to oral infection by Weeter's cultures. Cotton and Buck¹⁵ found that boars and pregnant sows could be infected regularly with *Brucella abortus* (*suis*) through the conjunctiva. The organism was recovered by inoculating guinea-pigs with the blood from 12 of 13 animals infected in this way. Five of the animals yielded positive blood cultures as long as 6 to 7 weeks following infection; positive blood cultures on the other 7 animals were obtained not longer than 2 to 4 weeks following infection. *Brucella* was recovered from the liver of 1 sow killed 102 days after infection. The organism was not recovered at autopsy from another sow killed after 102 days, nor from a sow that

died 48 days following infection. Feldman and Olson¹⁶ isolated brucella from 11 of 24 swine with spondylitis. These animals apparently were symptomless and showed no lesions in other parts of the body than the spine. Feldman and Olson¹⁷ also isolated brucella by guinea-pig inoculation from 2 of 102 apparently normal swine. The organisms were recovered from the lymph nodes on the head and in the anterior cervical region; they were also obtained from an abscess of the spermatic cord of one of the animals and from the spleen of the other animal. Gilman, Milks and Birch¹⁸ passed bovine strains of brucella through a series of hogs in experiments which demonstrated the failure of such animal passage to cause bovine strains to assume the characteristics of the porcine type. In the course of these investigations, large doses of organisms were administered intravenously to 18 sows. Brucella was not recovered by guinea-pig inoculation from 2 of the animals, killed at the end of 103 and 59 days respectively. The remaining 16 sows were killed from 21 to 57 days following infection. Brucella was recovered from the lymph nodes of all 16 animals, from the spleen in 4, from the liver in 1, from the ovaries and uterine wall in 4, and from the mammary gland in 4; in no instance was the organism recovered from the blood. Feldman and Olson¹⁹ introduced *Brucella suis* into swine by way of the conjunctiva, intravenously, orally and subcutaneously, and sacrificed the animals 319 days following infection. They found no lesions in these animals and failed to recover the organism by guinea-pig inoculation. From this experiment these workers concluded that swine apparently possess considerable natural resistance to experimental infection with one strain of brucella of porcine origin.

The experience of previous investigators of brucellosis in the dog and the hog thus indicates (1) that both the dog and the hog appear to possess considerable natural resistance to experimental infection with brucella, (2) that positive agglutination tests are not necessarily indicative of infection that can be demonstrated by recovery of brucella from the animal, and (3) that when the organisms are recovered, they are most frequently found in "integral parts of the reticulo-endothelial system" (van der Hoeden¹¹).

MATERIALS AND METHODS

In the present study, our procedures and observations were as follows:

Two strains of *Brucella suis* were selected for inoculation into the test animals: Strain A was isolated from the spleen of a naturally infected hog and was highly virulent for guinea-pigs, regularly producing

gross lesions and often death within 3 to 4 weeks after intraperitoneal inoculation. Strain B was obtained originally at autopsy from a case of Hodgkin's disease; at the time of these experiments, its virulence for guinea-pigs was slight.

Repeated inoculations of the test animals were made at intervals of from 7 to 21 days. In each instance, a freshly prepared bacterial saline suspension from a 48-hour agar slant culture standardized by means of the photronreflectometer was used.

Blood samples were cultured in Bacto-tryptose* broth with subculture to Bacto-tryptose sheep blood agar slants. Tissues obtained at autopsy were ground with sterile alundum and physiological saline solution to produce suspensions; small portions of these were streaked on Bacto-tryptose sheep blood agar plates, the remainder being added to poured plates of the same medium. The tissues selected for culture are noted in Tables I and II. In every instance, lymph nodes from at least two different sites were studied.

EXPERIMENTS

Dogs

Six male and 3 female adult mongrels were selected. Each appeared to be in good condition and weighed about 10 Kg. Preliminary brucella agglutination tests were negative, and brucella opsonocytophagic readings were within normal limits. Each animal was given an inoculum of 10 billion organisms at each injection. Four animals received repeated doses of *Br. suis* (strain B) intravenously; 1 animal, *Br. suis* (strain A) intravenously; 3 animals, *Br. suis* (strain B) intraperitoneally; and 1 animal, *Br. suis* (strain A) intraperitoneally. The dogs receiving *Br. suis* (strain B) were inoculated every 7 days for 105 days (except when it seemed doubtful that the animal would survive an inoculation), and every 21 days thereafter. The 2 dogs receiving *Br. suis* (strain A) were inoculated at 1, 14 and 34 days. The duration of the experiment for each animal is recorded in Table I. All dogs were bled from the jugular vein at frequent intervals for blood culture, brucella agglutination and opsonocytophagic tests.

The bacteriological findings are summarized in Table I and, together with the immunological data, are discussed later.

Swine

Six male and 2 female hogs, 6 weeks of age, were selected from a single litter. Preliminary brucella agglutination tests and blood cultures of both test animals and parent sow were negative. Preliminary

* Product of Difco Laboratories, Inc., Detroit, Mich.

TABLE I
Experimental Data for Dogs, Including Results of Blood and Tissue Cultures

Dog no.	1	4	5	2	10	9	3	6	7
<i>Brucella suis</i>									
Strain received	B	B	B	B	A	A	B	B	B
Inoculations*	21/i.v. 12/27	28/i.v. 23/34	4/i.v. 3/5	35/i.v. 18/42	3/i.v. 3/5	3/i.p. 1/5	39/i.p. 4/41	39/i.p. 0/43	33/i.p. 8/34
Blood cultures†									
Duration (in days)	198	261	38	487	216	186	461	454	398
Fate of animal	Died	Died	Died	Killed	Killed	Killed	Killed	Killed	Killed
Days between last inoculation and death	14	7	8	225	182	152	143	136	132
Days since last positive blood culture	7	7	1	233	101	152	270	None	231
Autopsy cultures:‡									
Spleen	+	+	+	—	—	—	—	—	—
Liver	+	+	0	—	—	—	—	—	—
Kidney	+	+	0	+	—	—	—	—	—
Lymph nodes	+	0	0	+	+	+	—	—	—
Testis	+	+	+	—	0	—	0	—	—
Lung	—	—	0	0	0	0	0	0	0

* Numerator refers to the number of inoculations given, denominator to the route of inoculation.
i.v. = intravenous; i.p. = intraperitoneal.

† Numerator refers to the number of positive blood cultures obtained, denominator to the total number of blood cultures done.

‡ 0 = not cultured; + = positive for brucella; — = negative for brucella.

opsonocytophagic indices of the test group ranged from 2.5 to 8.5. During the experiments all animals were bled at frequent intervals from the tail, the femoral vein, or the jugular vein for blood cultures, brucella agglutination and opsonocytophagic tests.

At intervals of from 4 to 8 days for 98 days, hogs 1 to 6 were inoculated intraperitoneally with *Br. suis* (strain B) in doses started at 70 million and increased by small amounts to 30 billion organisms. Beginning with the 15th week of the experiment, intravenous inoculations of 30 billion organisms were administered to hogs 1 to 5 every 7 to 14 days for the next 112 days, every 21 to 35 days thereafter. By means of a rubber catheter fitted with a syringe, hog 7 was given orally 7 doses of 30 billion *Br. suis* (strain A) organisms at 1, 6, 27, 42, 48, 70 and 111 days. Hog 8 received the same doses intravenously with omission of the 42nd day.

The bacteriological findings are summarized in Table II.

DISCUSSION

Nine dogs were subjected to large and repeated inoculations of *Br. suis*, 5 animals receiving the organisms intravenously and 4 intraperitoneally. The 5 dogs receiving *Br. suis* intravenously showed a high percentage of positive blood cultures when tested from 6 to 14 days following an inoculation; blood samples taken after 14 days yielded no growth, except in one instance (dog 10). Three of the 4 dogs receiving *Br. suis* intraperitoneally occasionally had positive blood cultures, 20 days being the longest observed period between inoculation of organisms and recovery of brucella from the blood. The greater incidence of positive blood cultures in the intravenous group is striking (Table 1): 42.9 per cent (18 of 42) to 67.6 per cent (23 of 34) positive, as contrasted to 0 to 23.5 per cent (8 of 34) in the intraperitoneal group.

No such difference was observed in agglutination and opsonocytophagic tests. In all of the dogs, regardless of route of inoculation or strain of *Br. suis* employed, the agglutination titer for *Brucella abortus* (456) rose to 1:10,240 or higher within 7 to 14 days following the first inoculation and remained at that level until termination of the experiment. The opsonocytophagic indices also rose within 7 to 14 days following the first inoculation and tended to remain high throughout the experiment. Wide individual variations were observed from week to week, but these could not be correlated in any way with the course of the infection. Two control animals, each of which was given three intravenous inoculations of 10 billion heat-killed *Brucella suis* (strain B) organisms at 1, 14 and 35 days, showed a similar

TABLE II
Experimental Data for Hogs, Including Results of Blood and Tissue Cultures

Hog no.	2	3	5§	1	4	8	6	7
<i>Brucella suis</i> strain received	B	B	B	B	B	A	B	A
Inoculations* (i.p. or p.o.)	15/i.p.	14/i.p.	13/i.p.	15/i.p.	14/i.p.	—	14/i.p.	7/p.o.
Blood cultures in above period†	0/7	0/7	0/6	0/7	0/7	—	0/5	0/6
Subsequent inoculations (i.v.)*	17/i.v.	17/i.v.	18/i.v.	14/i.v.	18/i.v.	6/i.v.	—	—
Subsequent blood cultures†	9/17	7/17	9/17	12/14	7/17	2/6	—	—
Duration (in days) of experiment	436 Killed	411 Killed	447 Killed	245 Killed	424 Killed	242 Killed	98 Died	240 Killed
Days between last inoculation and death	117	92	128	10	105	131	7	129
Days since last positive blood culture	180	133	169	0	168	208	None	None
Autopsy cultures:‡								
Spleen	—	—	—	+	—	—	—	—
Liver	—	—	—	+	—	—	—	—
Kidney	—	—	—	—	—	—	0	—
Lymph nodes	—	—	—	+	+	+	—	—
Testis	—	—	—	—	0	+	0	0
Lung	0	0	0	+	0	0	0	0

* Numerator refers to the number of inoculations given, denominator to the route of inoculation. i.p. = intraperitoneal; p.o. = per os; i.v. = intravenous.

† Numerator refers to the number of positive blood cultures obtained, denominator to the total number of blood cultures done.

‡ 0 = not cultured; + = positive for brucella; — = negative.

§ Spleen culture positive for brucella at operation on 87th day of experiment.

rise in brucella agglutination titer and opsonocytophagic index during the 157-day period in which they were followed.

At autopsy brucella was recovered from all of the dogs in the intravenous group and from 1 dog* in the intraperitoneal group. Our chief purpose was to determine, if possible, first, how long the organisms remain in the tissues when they are no longer demonstrable in the peripheral blood, and, second, under such circumstances, in what tissues they are most frequently found. Three dogs (nos. 1, 4 and 5) of the intravenous group died on the 198th, 261st and 38th day respectively. In all three instances, the last inoculation and the last positive blood culture occurred too recently to make the positive autopsy culture significant. Three dogs (nos. 3, 6 and 7) of the intraperitoneal group, killed on the 461st, 454th and 398th day respectively, yielded no positive cultures at autopsy. The remaining 3 dogs, 2 (nos. 2 and 10) of the intravenous group and 1 (no. 9) of the intraperitoneal group, were killed on the 487th, 216th and 186th day respectively, and the organism was recovered from each dog at autopsy. In these three instances, a significantly long period of time had elapsed since the last inoculation of organisms or the last positive blood culture (225, 101 and 152 days respectively). So, in these 3 dogs, the tissues from which the organisms were isolated at autopsy are of interest. Spleen, liver, kidney and lymph nodes were cultured in each instance, as well as testis from 2 of the 3 animals. Brucella was recovered from lymph nodes of all 3 dogs. The only other positive culture was obtained from the kidney of dog 2 (Table 1).

Essentially the same experiment was performed on hogs, using repeated inoculations of *Br. suis*. Of the 8 hogs employed, 1 was inoculated orally, 1 intraperitoneally, 5 intraperitoneally initially and then intravenously, and 1 intravenously. Blood cultures on the intraperitoneal and intraperitoneal-intravenous groups were uniformly negative during the 98-day period of intraperitoneal inoculations. During this time, however, on the 87th day of the experiment, 8 days after the 13th intraperitoneal inoculation of organisms, a culture of the spleen obtained at operation from 1 animal (hog 5) was positive for *Br. suis*; cultures of the liver, the peritoneal cavity, a mesenteric lymph node and the bile, made at the same time from this animal, were negative.

* Dog 9 was the one animal of the intraperitoneal group of 4 to be given *Br. suis* (strain A). This was the only evidence encountered to indicate greater virulence of *Br. suis* (strain A) over *Br. suis* (strain B) for either the dog or the hog, despite the marked difference known to exist between the two strains when inoculated into guinea-pigs. The lack of further evidence was particularly surprising in the experiments on the hog, inasmuch as strain A was isolated from a naturally infected hog and might have been expected to be highly virulent when re-introduced into the natural host.

Blood cultures from the orally inoculated animal (hog 7) were uniformly negative. In the group of 6 hogs subsequently receiving intravenous inoculations, the percentage of positive blood cultures was high (Table II): 33.3 per cent (2 of 6) to 85.7 per cent (12 of 14) as contrasted to none obtained during intraperitoneal or oral inoculation. As in the dogs, all cultures made more than 21 days following an inoculation were negative.

Within 40 to 55 days following the first inoculation, all 8 of the animals except hog 7 possessed agglutination titers for *Brucella abortus* (456) of 1:10,240 or higher. These titers were maintained throughout the experiment with two exceptions: The final readings done on hogs 3 and 5 showed titers of 1:5,120 and 1:2,560 respectively, and hog 7 receiving *Brucella suis* (strain A) orally possessed titers ranging irregularly from 1:320 to 1:5,120 from the 7th day until termination of the experiment. Opsonocytophagic indices of all of the animals were variable and not significantly elevated at any time.

The duration of the experiment for each animal is recorded in Table II. No positive cultures were obtained at autopsy from the 2 animals (hogs 6 and 7) receiving inoculations intraperitoneally and orally respectively. Positive autopsy cultures were obtained from 3 of the 6 intravenously inoculated animals.

As in the dogs, we had few animals which could be used to determine the presence of organisms in tissues when they were no longer demonstrable in the peripheral blood. Hog 1 of the intraperitoneal-intravenous group, killed on the 245th day, had a positive blood culture when sacrificed. Two animals (nos. 4 and 8) yielded positive autopsy cultures when killed on the 424th and 242nd day respectively. Both had received intravenous inoculations, their last inoculations or positive blood cultures occurring 105 and 131 days respectively before death. Spleen, liver, kidney and lymph nodes were cultured in each instance, as well as a testis of hog 8. Both animals (nos. 4 and 8) yielded positive lymph node cultures. In addition, brucella was recovered from the testis of hog 8.

CONCLUSIONS

1. *Brucella* usually disappears from the blood stream of both dog and hog within 1 to 3 weeks after an inoculation of organisms, but it can be recovered from the tissues in some instances from 3 to 7 months later.

2. In the absence of a positive blood culture and of all evidences of clinical infection at the time of autopsy, from inoculated dogs and hogs brucella was recovered most frequently from lymph nodes.

In an equal number of instances, the organism was not recovered from any site.

3. When *Brucella suis* is introduced intravenously into dogs and hogs, infection is frequently established, as shown by repeated recovery of the organism from the blood during life or from the tissues at autopsy; but animals so infected may present no clinical evidence of disease. It is extremely difficult to establish infection (demonstrable by recovery of brucella from the animal) by intraperitoneal inoculation. Infection of one hog by the oral route could not be confirmed by recovery of the organism.

4. The brucella agglutination titers of both dog and hog tend to rise early in the course of experimental inoculation with brucella and to remain high. Furthermore, the agglutination titer apparently is not materially influenced by the route of inoculation or the strain of *Br. suis* employed for inoculation; nor was any difference observed in this connection when heat-killed organisms were used instead of live organisms.

5. The brucella opsonocytophagic indices of both dog and hog were variable and could not be correlated in any way with the course of the experimental infection.

6. No striking difference in virulence between the two strains of *Br. suis* employed for the dog and the hog was observed despite the fact that for the guinea-pig the animal strain A was highly virulent and the human strain B only slightly so.

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THE EFFECT OF THE LEUKOCYTOSIS-PROMOTING FACTOR ON THE GROWTH OF CELLS IN THE BONE MARROW*

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Earlier studies made by me and my associates¹⁻³ have demonstrated the existence of a leukocytosis-promoting factor in numerous types of inflammatory exudates derived from various animal forms, including man. The presence of this factor offers a reasonable explanation for the mechanism of leukocytosis accompanying numerous inflammatory and infectious processes, for upon introduction of the exudative material into the blood stream of dogs or rabbits there follows, within a few hours, a discharge of immature leukocytes into the circulation.¹ This part of the study has recently been confirmed in rabbits by Reifenstein, Ferguson and Weiskotten.⁴ The prompt effect on the leukocyte level cannot be elicited by normal blood serum, bacterial suspension, nucleic acid, adenosine, or histamine.¹

Heating the exudate to a temperature of about 60°C. inactivates it.¹ Furthermore, dialysis indicates that the leukocytosis-promoting factor is evidently nondiffusible.² These findings have suggested the protein nature of the factor. Thereupon, fractionation with ammonium sulfate at various concentrations has been undertaken.⁵ The active factor has been isolated as a pseudoglobulin.⁵ In the earlier technic of extraction the euglobulin fraction of exudates was often retained with the pseudoglobulin. Furthermore, the injection of such fractions (as well as the untreated exudate) into the blood stream of dogs was frequently followed by a transient leukopenia which was subsequently replaced by a leukocytosis. The initial drop in the level of circulating leukocytes has recently been found to be referable to the presence of an injury factor in exudates, capable *per se* of explaining the fundamental pattern of cellular damage in inflammation.⁶ This factor is found to be associated with the euglobulin fraction of exudates. To it the term "necrosin" has been assigned.⁶ Besides inducing severe tissue injury accompanied by lymphatic blockade, necrosin, introduced into the circulation, is followed by a marked leukopenia. The polymorphonuclear leukocytes seem to be obliterated from the circulation leaving a differential leukocyte count characterized by a relative lympho-

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cytosis. It is my opinion that the liberation of sufficient necrosin at the site of injury may offer a reasonable explanation for the leukopenia accompanying some infectious processes. These studies have been reported *in extenso* in a separate communication.^{6a} At any rate it is clear that the initial leukopenia associated with the introduction of the leukocytosis-promoting factor can be readily disposed of by preliminary removal of the euglobulin fraction from exudates.

The present scheme adopted for the extraction of the leukocytosis-promoting factor can be briefly summarized as follows:

Exudate or cell-free exudate



$(\text{NH}_4)_2\text{SO}_4$ at $\frac{1}{3}$ saturation and centrifuge



↘ precipitate (euglobulin or "necrosin")

Supernatant



$(\text{NH}_4)_2\text{SO}_4$ at $\frac{1}{3}$ saturation, or even $\frac{1}{2}$ saturation



Dialyze precipitated mixture for 16 to 24 hours against tap H_2O



Reprecipitate residual nondiffusible material at $\frac{1}{2}$ saturation
 $(\text{NH}_4)_2\text{SO}_4$



Centrifuge



↘ supernatant

Pseudoglobulin precipitate taken up in distilled H_2O



Dialyze against tap H_2O until free of $\text{SO}_4^{=}$



Residual pseudoglobulin (leukocytosis-promoting factor)

The leukocytosis-promoting factor can be readily desiccated in a vacuum oven at about 35°C . or it can be dried by freezing in a Flosdorf-Mudd apparatus.⁷ The active material appears in the purified fractions as a whitish, rather brittle powder but which, with some adhering impurity, may have a trace of light brown color. The desiccated material is stable. When kept in a refrigerator for about 6 months it has been found unimpaired in its biological potency.

In the present state of purification the leukocytosis-promoting factor

is capable of inducing, within several hours, a rise in the number of circulating leukocytes ranging from about 70 to 300 or even 400 per cent above the original basal level. The effect is striking when it is recalled that, within the same interval, studies on the normal variation of the leukocytic level of dogs indicate an average maximum rise of about 25 per cent.^{1,5}

As previously stated, the leukocytosis-promoting factor is not recovered from normal serum. On the other hand, the material is readily extracted from the serum of an animal with a concomitant acute pleural inflammation, thus indicating that the effective pseudoglobulin probably reaches the bone marrow via the circulating blood stream.⁸ My earlier studies¹⁻³ have indicated that the developing leukocytosis is referable to a discharge of immature leukocytes into the circulation. This fact suggests that the leukocytosis-promoting factor acts directly on the hematopoietic tissue of the bone marrow. Is the effect merely due to an outpouring of already formed cells from the marrow, or does the substance likewise stimulate a corresponding growth or hyperplasia of myeloid elements?*

The present study represents a compilation of data on the effect of the leukocytosis-promoting factor on the bone marrow of dogs.

EXPERIMENTAL FINDINGS

Exudative material was obtained either from dogs or from human patients. In dogs inflammation was induced, as described in earlier communications,^{9,10} by the introduction, under nembutal anesthesia, of about 1.5 cc. of turpentine into the right pleural cavity. The leukocytosis-promoting factor was extracted as has been described. The concentration of material injected ranged from about 13 mg. to slightly over 100 mg., dissolved in physiological saline solution. In some of the experiments a single administration of the material into the circulation was employed. In other animals two or three injections were performed several hours or 1 day apart. The animal was sacrificed usually 1 or 2 days after the introduction of the leukocytosis-promoting factor. A thorough necropsy was performed. Samples of the bone marrow were obtained from the femur and from a rib. The tissue was fixed in a 10 per cent formaldehyde solution or in Zenker's fluid and acetic acid. The preparations were stained with hematoxylin and eosin or with Giemsa's stain. The bone marrow of several dogs with pleural inflammation was also studied. One animal was injected intravascu-

* Growth as used here is clearly synonymous with the term hyperplasia. It refers to an increase in the number of cellular elements in the bone marrow. This may be adequately considered as a specific growth reaction of hematopoietic tissue.

larly with the whole exudate and its marrow was subsequently fixed and stained. Two untreated dogs were sacrificed in order to obtain normal marrow. Finally, two control animals were injected with the pseudoglobulin fraction extracted from normal serum and their bone marrow was subsequently examined. The leukocytosis-promoting factor was, in several instances, recovered from exudates obtained from either the pleural or the peritoneal cavity of human patients. The material was then injected into dogs and the bone marrow subsequently studied.

The essential results of the observations appear in Table I. It is

TABLE I

The Effect of the Leukocytosis-Promoting Factor on Bone Marrow Hyperplasia

Dog no.	Type of material injected into the circulation or presence of pleural inflammation	Effect on the marrow (extent of hyperplasia indicated by number of plus signs)	Maximum increase in the number of circulating leukocytes as a result of injection of the LPF,* exudate, or serum pseudoglobulin
			per cent
7-64	LPF*	++	127.2
7-48	LPF	++	70.7
7-65	LPF	Trace to +	137.0
7-46	LPF†	++	170.4
7-49	LPF‡	+++	412.8
7-55	LPF‡	+++	291.4
		Average increase in the number of leukocytes	201.6
4-43	Exudate	++	68.5
7-59	Pleural inflammation	++	
7-39	Pleural inflammation	+++	
7-62	Pleural inflammation	Trace to +	
7-54§	Pleural inflammation	o	Marked leukopenia, W. B. C. of 2,450
7-50	Pseudoglobulin of serum	o	30.0
7-52	Pseudoglobulin of serum	o	69.5¶
		Average increase in the number of leukocytes	49.8
7-57	Control—no injection	o	
7-41	Control—no injection	o	

* LPF = leukocytosis-promoting factor.

† Leukocytosis-promoting factor obtained from human exudate; in other experiments the material was obtained from dogs.

‡ A single injection of only 13.5 mg. of LPF made. Other animals received much larger quantities of the material, either as previously desiccated or as the fluid fraction.

§ In this animal the inflammation in the pleural cavity was accompanied by a severe leukopenia in the circulation; the bone marrow was correspondingly found to show no signs of hyperactivity.

|| In the animals with pleural inflammation no observations were taken on the degree of ensuing leukocytosis; but previous studies¹ have indicated an average increase of 121.3 per cent in the leukocyte level of animals with similar pleural inflammation.

¶ This figure is somewhat elevated for the effect of normal serum pseudoglobulin on the leukocytic level.⁷ It is to be noted, however, that the serum which was slightly hemolyzed was originally obtained from a dog (7-51) that had received a single injection of leukocytosis-promoting factor into its circulation several days previous to withdrawing the sample of blood utilized in the present dog (7-52).

clear from the data that in dogs an acute pleural inflammation *per se* induces hyperplasia of the marrow. This is characterized primarily by active proliferation of the myeloid elements as well as of the megakaryocytes. This picture is similar to that recently described by Williams¹¹ on the marrow of patients afflicted by a pneumonic process. The effect on the marrow is precisely the same following the injection of the whole exudate. But it is important to note that the same picture with even a more marked hyperplastic response is observed following the injection of the leukocytosis-promoting factor. The usual fat of the femoral bone marrow is now almost completely replaced by an actively hyperplastic marrow. The myeloblasts, myelocytes and megakaryocytes seem to form the predominant cell types. The effect is illustrated in Figure 1. On the other hand, either normal marrow or the marrow of animals previously injected with the pseudoglobulin recovered from normal serum fails to manifest any hyperplastic response (Fig. 2). Another evidence of the proliferative effect caused by the leukocytosis-promoting factor on the myeloid tissue of the bone marrow is revealed by observing, at least in some of the preparations, a considerable number of mitotic figures. The number of megakaryocytes, as previously mentioned, may be markedly elevated.

The strikingly high potency of the leukocytosis-promoting factor in inducing in the blood stream an average increase in the leukocyte count of 201.6 per cent (Table 1) is doubtless referable to the dissociation of the leukopenia-inducing euglobulin fraction (necrosin) from the active pseudoglobulin (leukocytosis-promoting factor) of exudates. In earlier studies^{1, 3, 5} the failure to take this factor into consideration has yielded definitely weaker fractions of leukocytosis-promoting factor.

DISCUSSION

The observations reported previously and in the present communication indicate that the leukocytosis-promoting factor recovered as a pseudoglobulin from inflammatory exudates not only offers a reasonable explanation for the mechanism of leukocytosis associated with inflammatory processes, but it is also clear that this globulin fraction induces hyperplasia or growth of myeloid cells as well as megakaryocytes in the bone marrow. This effect is readily observed both in the femoral bone marrow and in the marrow of ribs. This double potentiality of inducing an acceleration of growth and a discharge of leukocytes into the circulation may be closely linked. It is conceivable that the promptness of appearance of a leukocytosis in the circulation is conditioned by the activity of the bone marrow at the time of injec-

tion of the leukocytosis-promoting factor. A relatively aplastic marrow would probably tend to show at first a rather sluggish response to the presence of this active protein as compared to an already active marrow. This fact may perhaps account in part for the variation in time response obtained in different animals with the same fraction of the material. For instance, in one dog the leukocytosis may be elicited in about 2 hours, whereas in another animal the same material may induce a similar effect only after 12 to 24 hours. There is some suggestive evidence that both growth and maturation of the leukocytes are not only hastened in the marrow but that the maturation process may perhaps be also accelerated in the circulating leukocytes. Whether, however, this enhanced maturation actually occurs outside of the hematopoietic tissue remains to be determined with greater precision. Such studies are now in progress and will form the subject of future reports. In brief, the observations of the present experiments clearly indicate the isolation of a pseudoglobulin from inflammatory exudates which seems to have a direct and specific effect on the growth of some of the cells of the bone marrow without producing any detectable or injurious effect on other tissues. This finding may have definite clinical implications. It is known that the prognosis of numerous infectious processes depends, to some extent at least, on the level of circulating leukocytes. The injection of the leukocytosis-promoting factor, by promoting hyperplasia of the marrow and a discharge of leukocytes into the circulation, may prove to be of clinical value. It is also conceivable that this active biological substance may be of aid in agranulocytic and other aplastic conditions of the marrow. Furthermore, in view of its specific effect in hastening the growth of myeloid elements, it remains to be seen what effect the leukocytosis-promoting factor may have on the course of experimental leukemia. Such a study is now in progress.

CONCLUSIONS

An inflammatory exudate injected into the blood stream of dogs has been shown to induce a rise in the number of circulating leukocytes. The effect is due to the presence of a leukocytosis-promoting factor liberated at the site of a recent injury. This factor has been recovered as a pseudoglobulin from exudates. It penetrates into the blood stream from the site of inflammation and thus reaches the bone marrow. It induces a discharge of immature leukocytes from the marrow into the circulation. This fact offers a reasonable explanation for the mechanism of leukocytes accompanying numerous inflammatory processes.

The leukocytosis-promoting factor has also a direct and specific

growth effect on some of the cells of the bone marrow. Its injection into dogs is followed by a marked hyperplasia of the myeloid elements of the bone marrow. This is also accompanied by an augmentation in the number of megakaryocytes. The various implications of the two potentialities of the leukocytosis-promoting factor in being capable of eliciting a discharge of leukocytes into the blood stream together with a hyperplasia of granulocytic cells in the bone marrow are pointed out.

I wish to express acknowledgment to Mr. M. Kadish and Miss Carolyn Clemente for assistance in the course of this study, and to Miss E. L. Sweet for the photomicrographs.

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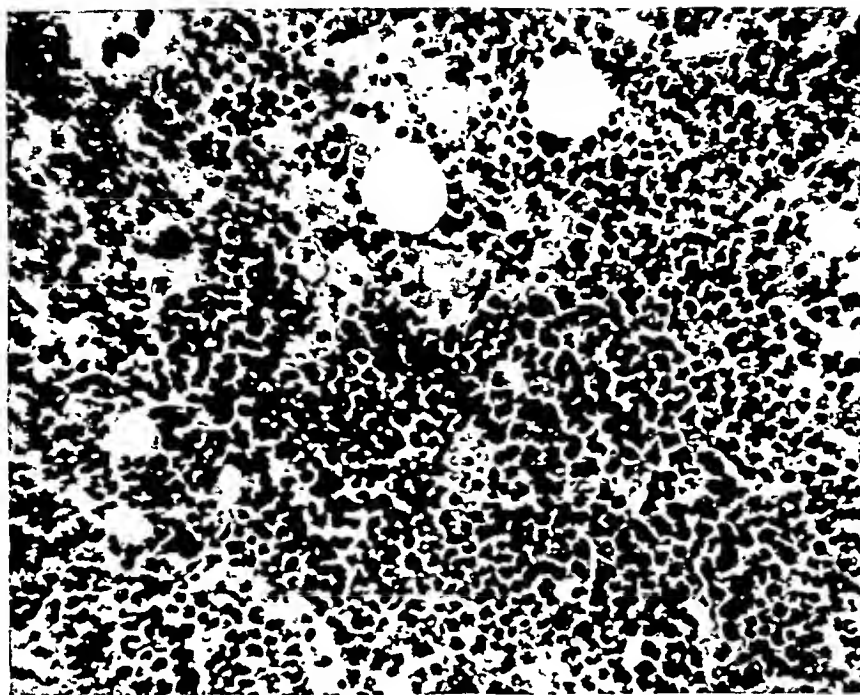
[Illustrations follow]

DESCRIPTION OF PLATE

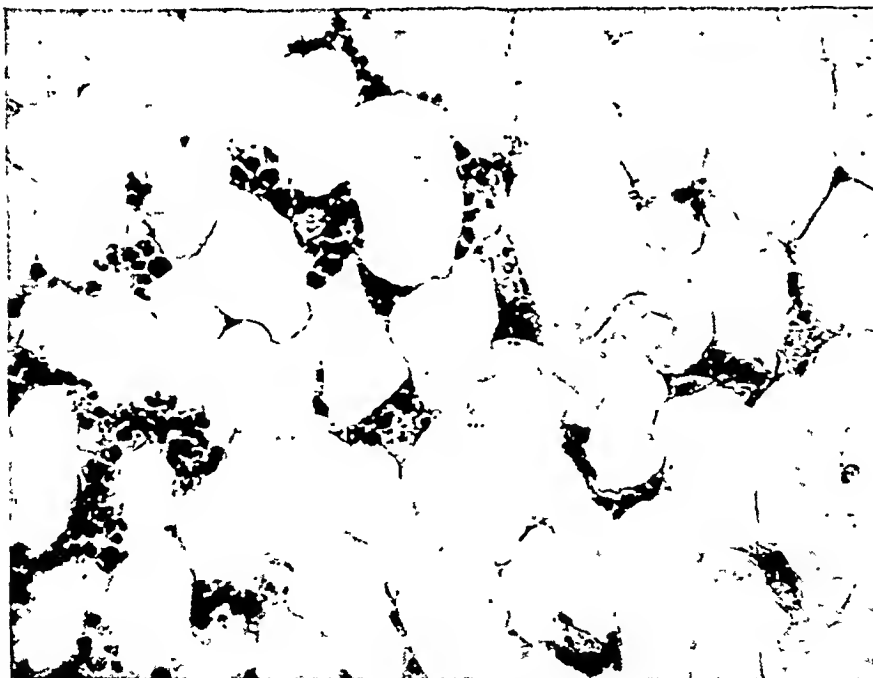
PLATE 125

- FIG. 1. Femoral bone marrow of dog 7-49, 2 days following a single injection of 13.5 mg. of the leukocytosis-promoting factor. The hyperplasia is striking. $\times 330$.
- FIG. 2. Femoral bone marrow of dog 7-50, 2 days following an injection of 14.5 mg. of pseudoglobulin derived from normal blood serum. There is clearly no evidence of any hyperactivity. $\times 330$.

1



2



Menkin

Leukocytosis-Promoting Factor and the Bone Marrow



THE ANTERIOR PITUITARY GLAND IN WOMEN WITH CARCINOMA OF THE MAMMARY GLAND, WITH REPORT OF A CASE OF CHROMOPHOBE ADENOMA*

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The purpose of the present study was to determine whether the pituitary glands of women who had carcinoma of the mammary gland showed changes which might be interpreted as hyperestrogen effects, and thus, by a new approach, to gain knowledge on the causation of human breast cancer. Our interest in such studies was aroused by a chromophobe adenoma of the pituitary gland in a woman who had mammary cancer, and by the numerous reports from experimental laboratories dealing with relationships between estrogens, the pituitary gland and breast tumors in animals.

When estrogenic hormones are injected into mice and rats, tumors of the breast are induced under certain conditions. (See reviews and recent papers by Haagensen and Randall,¹ Geschickter and Byrnes,² Gardner,³ Lacassagne,⁴ Cramer,⁵ Greene and Brewer,⁶ Allen,⁷ and others.) The injection of estrogens into these animals may also produce changes in the anterior pituitary gland consisting of hyperplasia, vascular congestion and tumors (Hohlweg,⁸ Wolfe,⁹ Cramer and Horning,¹⁰ Zondek,¹¹ McEuen, Selye and Collip,¹² Burrows,¹³ Zondek,¹⁴ Perry and Lockhead,¹⁵ Lacassagne and Nyka,¹⁶ Weil and Zondek,¹⁷ Gardner,¹⁸ and others). There is some relationship, the exact nature of which is unknown, between estrogen and the tumors in the two glands. It seems to be well established that the ovarian hormones may be one factor in the causation of breast tumors in mice.^{19, 20}

In women, also, imbalance or excessive amounts of ovarian hormones have been suspected of being implicated in the causation and in the subsequent growth of breast cancer for the following reasons, among others:†

1. Mammary carcinoma has appeared in women who received estrogenic therapy in cases reported by Allaben and Owen,²¹ Auchincloss and Haagensen²² and Parsons and McCall.²³ A causal relationship has, however, not been proved by these cases. It has not been

* Received for publication, February 1, 1943.

† Complete bibliographies are not attempted. Generally only some of the recent papers, especially those having numerous references, are quoted. Also, many papers giving evidence which fails to confirm these claims are omitted. Thus the strongest possible position is presented, without rebuttal, and with the realization that the analysis is not critical.

shown that a higher percentage of women receiving estrogenic therapy develop breast cancer than of women of the same age who had no such therapy.

2. Women who have previously had oophorectomy develop less mammary gland cancer (1.5 per cent in 1,906 women) than women who have not (15.4 per cent in 1,011 women), according to Herrell.²⁴

3. There are probably close relationships of estrogens to cystic mastitis, and of the latter to breast cancer (Patey,²⁵ Warren,²⁶ Taylor,²⁷ Logie,²⁸ and others).

4. The incidence of a history of menstrual irregularity is high in patients with mammary cancer (Taylor^{29,30}).

5. The menopause occurs later in women who have breast cancer than in other women (Olch,³¹ Heiberg and Heiberg³²).

6. Seventy-five per cent of the cases of breast cancer occur in the period of greatest ovarian activity or within 5 years thereof (Olch,³¹ Taylor³³).

7. There is a high coincidence of breast and uterine tumors (Taylor,³⁴ Meigs³⁵).

8. Unmarried women have relatively more breast cancer than married women (Heiberg and Heiberg,³² Adair,³⁶ Bromeis³⁷).

9. The incidence of a history of faulty nursing is high in women with mammary cancer (Taylor,³³ Adair,³⁶ Bromeis,³⁷ Trout³⁸).

10. The incidence of a primary carcinoma in the second breast following removal of one breast for cancer is high (Trout,³⁹ Kilgore⁴⁰).

11. Improvement of premenopausal patients with mammary cancer, judged especially by bone metastases, has followed castration by surgery or sterilization by irradiation (Beatson,⁴¹ Ahlbom,⁴² Dresser,⁴³ Taylor,⁴⁴ Pohle⁴⁵).

12. Breast cancer may be stimulated by pregnancy (Bromeis,³⁷ Trout³⁹).

No one of these lines of evidence proves that estrogens are the immediate cause of human mammary gland cancer, and neither does their summation, although the sum is impressive. The accumulated observations emphasize the unusual status of these women from the hormonal point of view.

Further evidence might be found in the pituitary gland. If excessive amounts or imbalances in estrogens are factors in the causation of cancer of the human mammary gland, as well as in lower animals, perhaps the pituitary body will show changes resembling those seen in animals after injections of estrogens. Having access to human material, we have made a search for such changes.

Among thirteen pituitary bodies obtained from women who had

carcinoma of the mammary gland, one, to be described, had a large adenoma. It is possible that this represents the human counterpart of the pituitary tumors found in animals following injections of estrogens. The other twelve showed no obvious gross or microscopic deviations from normal, so that actual differential counts of the types of cells were made to detect small differences.

METHODS

Twelve pituitary glands from women with mammary carcinoma were fixed, generally in formaldehyde, and embedded in paraffin, after which sections $4\ \mu$ thick were cut in the midsagittal plane. In one case horizontal sections were used. The sections were stained by a variety of methods, including Gomori's chromium hematoxylin and phloxine;⁴⁶ Mallory's phosphotungstic acid hematoxylin, and his acid fuchsin, aniline blue, orange G methods; hematoxylin and eosin; Maximow's hematoxylin, eosin, azure; and Bailey's aniline fuchsin, acid violet; and his ethyl violet, orange G methods. The latter were used because some pituitary glands, not included here, had been fixed in Regaud's solution. The differential stains on these sections were not satisfactory, and this material eventually had to be discarded. The final cell counts were made, with one exception, on sections stained by Dr. Georg Gomori by his method.

Differential cell counts were made under oil immersion. Four thousand cells from each gland were counted, 2,000 being counted independently by each of the authors. Every cell was counted which contained a nucleus, and which a pointer fixed in one ocular touched in passing across the field. The counts were begun at one capsule and continued entirely across the gland to the opposite capsule. Generally four vertical and four anteroposterior runs, evenly spaced from each other, were required by each of us.

Sections not over $5\ \mu$ thick were essential for accurate counting to avoid under-counting of those chromophobe cells which had little cytoplasm. The overlap of the heavily stained cytoplasm of adjacent chromophils could lead to counting chromophobes as chromophils. Paraffin embedding was found to be superior to celloidin because it caused a slight retraction of the cytoplasm.

As controls, differential cell counts were made by similar methods on the pituitary glands of 15 women who died of tumors located in organs other than the mammary gland. The valuable data of Rasmussen^{47, 48} on normal pituitary glands supply standards and control data for work by others. However, because our material and our methods, of necessity, did not conform to his it was thought desirable

for this work to have its own controls. For instance, in both of our groups the patients did not die suddenly as did his but were ill for some time with malignant neoplasms. Our method of making counts from one midsagittal section was not ideal and it was deemed necessary to have a control group counted by the same method.

RESULTS

Gross Appearance. With the exception of the case which had an adenoma (to be described in detail), the pituitary bodies grossly appeared to be practically normal. In those instances in which weights or measurements were recorded, they were within the normal range. One showed a tiny tumor metastasis in the posterior lobe on microscopic examination.

Percentage of Chromophobes. The chromophobes averaged 47.5 per cent in the cases with carcinoma of the mammary gland and 48.2 per cent in the controls. These data are shown in Tables I and II. In Table III it can be seen that these differences are not significant. The individual variation was great. It ranged from 35 to 57 per cent in cases of carcinoma of the breast, and from 30 to 71 per cent in the controls.

TABLE I

Percentage of the Different Types of Cells in Anterior Lobe of Human Hypophysis in 12 Women with Carcinoma of the Mammary Gland

Cell type	Minimum	Maximum	Median	Mean and probable error	Standard deviation	Coefficient of variation
Chromophobe	35.3	56.7	48.5	47.5 ± 1.34	7.07	14.9
Acidophil	21.9	56.0	38.4	38.9 ± 1.84	9.65	24.8
Basophil	8.4	25.9	11.8	13.6 ± 0.96	4.98	36.6

TABLE II

Percentage of the Different Types of Cells in Anterior Lobe of Human Hypophysis in 15 Women with Miscellaneous Tumors

Cell type	Minimum	Maximum	Median	Mean and probable error	Standard deviation	Coefficient of variation
Chromophobe	29.8	71.5	49.8	48.2 ± 1.94	11.25	23.3
Acidophil	12.0	59.4	35.1	35.4 ± 1.87	10.85	30.6
Basophil	8.8	30.1	14.8	16.4 ± 6.35	36.88	224.8

TABLE III

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Women with Carcinoma of the Mammary Gland and Those with Other Tumors

Cell type	Carcinoma of the mammary gland (12 cases)	Miscellaneous tumors	Difference	Probable error of difference	Difference probable error of difference
Chromophobe	47.5 ± 1.34	48.2 ± 1.94	-0.7	2.35	0.2
Acidophil	38.9 ± 1.84	35.4 ± 1.87	+3.5	2.63	1.4
Basophil	13.6 ± 0.96	16.4 ± 6.35	-2.8	6.42	0.4

Percentage of Acidophils. The acidophils averaged 38.9 per cent in the breast cancer cases and 35.4 per cent in the control cancer cases. As is shown in Table III, these differences are not significant. Again, the individual variation was great, ranging from 22 to 56 and from 12 to 59 per cent, respectively, in the two groups.

Percentage of Basophils. The basophils averaged 13.6 per cent in the cases with carcinoma of the breast and 16.4 per cent in the control group. This difference is not significant, as is shown in Table III. The basophils also showed great individual variation, ranging from 8 to 26 per cent in the breast cancer cases, and from 9 to 37 per cent in the controls. Most of the counts in the control group fell between 7.4 and 18.0 per cent, but two were far greater: they were 30.1 and 37.1 per cent.

DISCUSSION

In Table III the results of the cell counts in the two groups of patients—carcinoma of the breast and miscellaneous tumors—are compared. In the last column the ratio of the differences is given. It is less than 1.5 with respect to each type of cell, showing that the differences are not significant. The relative proportions of the different types of cells in these two groups of patients, then, may be considered to be in the same range.

There remains, however, the necessity of comparing these two groups with counts from normal women. For this purpose the best data are those of Rasmussen.⁴⁷ In Table IV the cases of carcinoma of the breast are compared with Rasmussen's normal standards, and in Table V the miscellaneous tumor cases are similarly treated. It is seen that

TABLE IV

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Women with Carcinoma of the Mammary Gland and in Normal Women

Cell type	Normal females (Rasmussen's 94 cases)	Carcinoma of the mammary gland (12 cases)	Difference	Probable error of difference	Difference probable error of difference
Chromophobe	49.6 \pm 0.47	47.5 \pm 1.34	+2.1	1.43	1.5
Acidophil	43.4 \pm 0.56	38.9 \pm 1.84	+4.5	1.91	2.4
Basophil	7.0 \pm 0.20	13.6 \pm 0.96	-6.6	.98	6.7

TABLE V

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Women with Miscellaneous Kinds of Tumor and in Normal Women

Cell type	Normal females (Rasmussen's 94 cases)	Miscellaneous tumors (15 cases)	Difference	Probable error of difference	Difference probable error of difference
Chromophobe	49.6 \pm 0.47	48.2 \pm 1.94	+1.4	1.99	0.7
Acidophil	43.4 \pm 0.50	35.4 \pm 1.87	+8.0	1.94	4.1
Basophil	7.0 \pm 0.20	16.4 \pm 6.35	-9.4	6.35	1.5

the results are in the same range except for one comparison in each table. These require further discussion.

In the last column in Table IV, the ratio of the differences to the probable error of these differences, with respect to basophils, is seen to be 6.7. A ratio of 4 means that there is less than one chance in one hundred that the difference is due to random sampling. This increase in basophils, then, appears to be statistically significant. This ratio is still significant (5.3) if our results are compared with those given by Rasmussen for normal women over 50 years of age. The 12 women in our group of carcinoma of the breast averaged 58.8 years of age. Eight of them were over 50 years, so that this comparison is fair.

Although the data appear to demonstrate a real increase in the basophils in women who had carcinoma of the mammary gland over those in normal women, this conclusion should not be drawn until a larger series of cases is studied. Furthermore, our sampling of the different parts of the pituitary gland was less thorough than that by Rasmussen, so that the results cannot be compared precisely because the methods were different. Even if an increase in the basophils were established, the interpretation would be uncertain because increases in this cell have been reported in numerous nonneoplastic conditions.^{48, 49}

In the last column in Table V, the ratio of the differences to the probable error of these differences for acidophils is seen to be 4.1. Being over 4 this appears to be significant until the data are recalculated to compare with Rasmussen's figures for normal women over 50 years of age (average 61 years). When this is done the ratio falls to 2, a figure not statistically significant. These women with miscellaneous tumors averaged 47 years of age, and 7 of them were over 50 years. While this recalculation is not strictly justified, it casts considerable doubt on the importance of the figure of 4.1 for acidophils in Table V. The conclusion is that a reduction of acidophils has not been demonstrated beyond doubt in women with various types of tumors.

In mice, mammary gland tumors may be caused by several different factors, according to Bittner.^{19, 20} Although the causation of carcinoma of the mammary gland in women is less well known, it is possible that different combinations of factors might be operating in different cases. For example, the rôle of estrogenic hormones might be more important in premenopausal mammary gland cancer than in that which begins after the menopause. If this is true, the pituitary glands might be different in the various age groups. When our data were examined from this point of view no differences were revealed. The proportions

of the three types of cells were approximately the same in the 4 women who were under 50 years of age as in the 8 who were over that age.

Data on the condition of the pituitary gland in women with carcinoma of the breast were not found in the literature except in so far as they could be culled from general papers. No reports of differential counts were found although many changes, unsupported by evidence, were reported. The pituitary gland did not appear to be enlarged in the case of carcinoma of the breast in Wyeth's⁵⁰ tables. The average weight for his 8 cases was 0.878 gm. as contrasted with an average of 1.050 gm. for 3 women with carcinoma of the uterus, and an average of 0.811 gm. for 28 women with tumors of other types.

There is agreement among all observers that the chromophobes are the cells which are responsible for the enlargement of the pituitary gland in animals following estrogenic therapy.^{17, 51} On the basis of 12 cases reported here there is no evidence that the chromophobes are increased in women with carcinoma of the breast. The failure to demonstrate changes in the chromophobe cells does not exclude a hormonal factor in the causation of these mammary gland tumors because it is not known that the human being reacts in the same way as do rodents. Furthermore, changes in the pituitary gland are not found constantly in animals with estrogen-induced mammary gland tumors. Unfortunately, in the 3 human cases of carcinoma of the breast, described by others, which followed estrogenic therapy the condition of the pituitary gland was not stated. Such observations should be made if additional cases are discovered. Neither have changes in the pituitary gland been described in women with granulosa cell tumors of the ovary.

CASE OF CHROMOPHOBE ADENOMA OF THE PITUITARY GLAND

Although there were no visible enlargements and no changes in the chromophobe cells in the hypophyses of 12 women who had carcinoma of the mammary gland, as reported in the preceding section, a 13th case with this disease had a chromophobe adenoma. The question arises as to whether this is the human counterpart of the tumors seen in animals or whether it was coincidence.

Besides the changes in the pituitary gland which follow the injection of estrogens in animals, several observations related to the natural disease are of interest. Thus, Gardner, Strong and Smith⁵² described, in a mouse, the spontaneous occurrence of tumors in the mammary gland, the pituitary body and the ovaries. Wolfe, Bryan and Wright⁵³ found that chromophobe cells were generally more abundant in rats which had mammary tumors.

REPORT OF CASE

Mrs. R. P., white, aged 42, entered the University of Chicago Clinics on September 14, 1931, for the removal of a breast tumor which had been present for 4 months. The breast, containing a carcinoma, was amputated and the axillary lymph nodes, also containing carcinoma, were resected. The patient died of pneumonia 6 weeks after operation. Necropsy (by Dr. E. M. Humphreys) revealed small but widespread carcinoma metastases in the pleura, lungs, liver, adrenals, brain, and lymph nodes of the thorax and abdomen.

That part of the record which was possibly related to the pituitary gland was as follows: Her menses began at 12 years. They were painless, regular at 28 days, and lasted for 2 days with a moderate flow. She had married at 20 years of age, and had been pregnant six times, giving birth to six living children. In 1925, when she was 36 years old, she was knocked unconscious in an automobile accident, following which the menses stopped permanently. Two years later she was hospitalized for 3 months because of a polyarthritis. The only manifestations possibly related to enlargement of the pituitary gland were attacks of vomiting, and mental confusion.

At necropsy the pituitary gland was found to be enlarged to about three times its normal size. The sella turcica was enlarged and the clinoid processes were eroded. The gland was still encapsulated. It pressed upward against the floor of the third ventricle and compressed the optic chiasm and the optic nerves.

Microscopic examination of this tumor disclosed that it was composed of masses of cells with scant cytoplasm and fairly small oval nuclei. There were no mitotic figures. The tumor had little stroma. Special stains failed to reveal any cytoplasmic granules. The structure was compatible with the diagnosis of the small cell type of chromophobe adenoma.

There were no remarkable changes in the adrenals, ovaries, uterus, thyroid, thymus, pancreas, or remaining mammary gland.

COMMENT

It is difficult to make final interpretation of this case at the present time. The coexistence of an adenoma of the pituitary gland and carcinoma of the mammary gland may have been without significance, since the incidence of pituitary adenomas in routine autopsies has been stated to be 10 per cent.⁵⁴⁻⁵⁷ While true adenomas are occasionally encountered as incidental findings, this high figure is open to some question, because of the criteria used as to what constitutes an adenoma. In many pituitary glands areas are found which, at first glance, appear to be composed of one type of cell but more careful study reveals that there is an admixture of cells of other types.

It is impossible to attribute with assurance either the mammary gland carcinoma or the pituitary gland adenoma to an increased amount of estrogen because menstruation had stopped 6 years before

the tumors were recognized, and because the uterus showed no hyperplasia. Although elaborate theoretical explanations could be devised, they would not prove the points at issue.

A follow-up on persons who survived operations for chromophobe adenomas from the point of view of the incidence of subsequent development of carcinoma of the mammary gland has not been made on a large series of patients to our knowledge. Neither have we found a record of a high incidence of pituitary gland adenomas in cases of cancer of the breast. The incidence of subclinical pituitary gland adenomas is about the same in the two sexes, which is unlike the sex ratio of breast cancer. The incidence of adenomas of the pituitary body has been stated to be increased in cases of adenoma of the prostate gland, carcinoma of the pancreas, carcinoma of the rectum, and in carcinoma of the bronchus.⁵⁷

SUMMARY

Differential cell counts were made on the anterior lobes of the pituitary glands obtained from 12 women who had carcinoma of the mammary gland, and, as controls, on pituitary glands from 15 women who had tumors of other types. There were no important differences in the percentages of chromophobes, acidophils or basophils in these two groups.

When these cell counts were compared with those given by Rasmussen^{47,48} for normal women, the chromophobes and the acidophils were within normal limits while the basophils were increased. Since the size of the group was small, and the sampling of all parts of the pituitary glands was not thorough, this difference should not be accepted as final.

Although the chromophobe cells were not increased in 12 cases of carcinoma of the mammary gland, in a 13th case an adenoma, of chromophobe cell type, was found.

Thus, there was no evidence found in the 12 pituitary glands for hyperestrogen effects, although it is possible that the human pituitary gland does not respond to estrogens in the same way as does that of rodents. The adenoma, however, might possibly be considered as evidence for a hyperestrogen effect, although proof of this cannot be offered.

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DECIDUAL REACTIONS IN FALLOPIAN TUBES

HISTOLOGIC STUDY OF TUBAL SEGMENTS FROM 144 POST-PARTUM STERILIZATIONS *

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This report is based upon histologic examination of excised tubal segments from 144 consecutive post-partum sterilizations done at the Kapiolani Maternity and Gynecological Hospital during the 3-year period from 1940 to 1942 inclusive. It was prompted by the frequent observation, during the past 2 years, of a decidual reaction in such tubal segments. Consequently, all tubes removed at post-partum sterilizations during 1942 have been studied more carefully than is the usual custom with such specimens. In most cases sections were prepared at intervals through each tubal segment and in a number of instances complete serial sections were made. In addition, all sections of post-partum tubes removed during the preceding 2-year period were reviewed, although in most of these cases only one microscopic section through each tubal segment was available for examination. The tubal segments varied in length from 1 to 2 cm. and in all but 2 cases comprised the proximal (isthmian) portions.

One or both tubal segments from 17 (12 per cent) of these 144 cases exhibited decidual formation of varying extent and location. These cases are analyzed in Table I.

One-half of the patients making up this group of 144 cases were Hawaiian or part Hawaiian in nationality. The majority of the remainder were Japanese, Chinese, and Filipinas, and there were relatively few Caucasians. Eleven (64 per cent) of the 17 cases showing a tubal decidual reaction belonged to the Hawaiian or part Hawaiian group and there were only 3 Caucasians (2 of them Portuguese) in the remaining 6. The significance of this finding will be considered later.

The elapsed time in hours between delivery and operation varied from 0 (cesarean section) to 65. There was no constant correlation between the length of time between delivery and operation and the presence of decidual tissue or the amount of decidual cell involution.

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TABLE I
Analysis of 17 Post-partum Sterilizations in which Decidual Change was Found in the Excised Tubal Segments

Case no.	Race	Age	Para	Hours post-partum	Number of sections studied	Location of decidua	Amount of decidua	Involution	Serosal mesothelium
1	Pt. Hwn.	28	4	28	Serial	Mucosal and serosal	Great	Advanced	Cuboidal
2	Cauc.	26	5	20	1	Mucosal	Slight	Slight	Flat
3	Pt. Hwn.	26	4	60	4	Mucosal and serosal	Slight	Slight	Cuboidal
4	Pt. Hwn.	28	3	0	1	Serosal	Slight	None	Flat
5	Hwn.	24	4	47	1	Mucosal	Slight	None	Flat
6	Hwn.	33	7	44	1	Mucosal	Moderate	Moderate	Flat
7	Cauc.	30	2	57	2	Mucosal	Moderate	Moderate	Flat
8	Hwn.	33	4	12	Serial	Mucosal	Great	Slight	Cuboidal to columnar
9	Cauc.	30	2	0	1	Serosal	Slight	None	Cuboidal
10	Pt. Hwn.	25	4	58	1	Mucosal	Slight	Advanced	Flat
11	Pt. Hwn.	27	4	32	1	Mucosal	Moderate	Slight	Flat
12	Pt. Hwn.	34	2	0	Serial	Mucosal	Slight	None	Cuboidal
13	Jap.	38	6	8	1	Serosal	Moderate	Moderate	Cuboidal
14	Jap.	38	2	27	Serial	Mucosal	Great	Advanced	Cuboidal
15	Pt. Hwn.	25	4	20	1	Serosal	Slight	Moderate	Cuboidal to columnar
16	Jap.	28	4	65	100 μ apart	Serosal	Moderate	Moderate	Cuboidal with invaginations
17	Hwn.	32	10	21	100 μ apart	Mucosal and serosal	Moderate	Slight	Cuboidal

Hwn. = Hawaiian

Pt. Hwn. = Part Hawaiian

Jap. = Japanese

Cauc. = Caucasian (including Portuguese)

LOCATION OF DECIDUA

In 9 cases the decidual tissue occupied the mucosal folds, in 5 it was subserosal in location and in the remaining 3 it was found in both locations. The involved mucosal folds were swollen and distended by the decidual cells, and the covering tubal epithelium was, without exception, greatly flattened but always recognizable as a distinct layer (Figs. 2 and 4). The tips or free margins of the rugae showed the greatest change; toward the bases of the folds decidual cells were rarely found. Where the process was extensive the rugae appeared as pedunculated spherical masses attached to the wall by narrow pedicles (Fig. 1). Where involution was slight the decidual cells were well preserved structurally and presented the characteristic mosaic pattern seen in the endometrium (Fig. 4). The cells, however, tended to vary somewhat in size and shape and many of them were elongated and spindle-shaped.

The serosal decidua in general was less well developed than that in the mucosa, and there was even greater variation in the size and shape of the cells. In only one instance (case 1) was there a continuous mantle of cells surrounding the tube. In the remaining cases the cells existed in the form of small accumulations just beneath the mesothelium. The patches of decidua were most numerous in the region of the mesosalpinx and often existed in the form of small evaginations covered by flat serosal mesothelium (Fig. 5).

AMOUNT OF DECIDUA

In cases 1, 8 and 14 the decidual tissue was abundant, practically all of the mucosal folds in both segments showing this change, and serial sections in these 3 cases showed the decidual tissue to extend longitudinally throughout the entire length of the tubal segment (Fig. 1). In 6 cases the decidual formation was moderate in amount (less than one-half of the mucosal folds involved; two to four patches of decidua just beneath the mesothelium in those with serosal involvement). In 8 cases only slight decidual change was present. These exhibited from one or two cells up to one or two groups of cells either in the tip of a mucosal fold or in a patch beneath the serosa (Fig. 3).

DECIDUAL CELL INVOLUTION

In 9 cases there was no, or at most very slight, decidual cell involution; moderate or advanced involution was present in the remaining 8. Study of the latter group of cases yielded interesting data on decidual cell involution. The initial stage in this process is

the appearance of a large round vacuole in the center of the cell with displacement of the nucleus toward the periphery (Fig. 5). This is followed by additional large vacuoles resulting in complete disappearance of the cytoplasm and further peripheral displacement of the nucleus. At this stage the cell is large and appears as a ring form not unlike the signet ring cell of mucoid adenocarcinoma (Fig. 6). The nucleus is elongated or band-like and is located far to the periphery at one margin of the cell. The nucleus then disappears and the cell shrinks and becomes irregular, forming a faintly staining small tear-drop or crescent-shaped structure which soon vanishes.

The end result of this process is the formation of a cyst or cysts at the decidual cell site. As each cell involutes it leaves behind the space which it formerly occupied. Thus in those cases showing involution, the decidual cells were loosely arranged, each separated from its fellow by a clear space, and the remaining cells were in various stages of the degenerative process already outlined (Fig. 2). In several locations this process was so extreme that the bulbous mucosal fold had been largely converted into a cyst in which only a few decidual cell remnants could be identified. A sprinkling of lymphocytes was usually present in the involuting areas.

SEROSAL MESOTHELIUM

In 11 of the 17 cases the serosal mesothelium was hyperplastic in nature and cuboidal to columnar in appearance. The cells had undergone peculiar rounded swelling due to increase in the cytoplasm (Figs. 5 and 7). The nuclei were round or oval and were located centrally. In 1 case invaginations into the underlying tissue were observed. Serosal hyperplasia was also found in many of the cases which failed to exhibit decidual tissue. The serosal mesothelium over the patches of decidua could usually be recognized as an intact and separate layer although it was greatly flattened in these locations (Fig. 5).

HISTORICAL ASPECTS

It has long been known that decidual reactions occur in many locations remote from the uterus. Novak¹ mentioned that such reactions have been described on the surface of the uterus, the anterior surface of the rectum, the floor of the cul-de-sac, the omentum, the ovary, the appendix, the cervix, the vagina and the peritoneum of the small intestine. Weller,² in 1935, reviewed the literature on this subject and discussed the significance of the ectopic decidual reaction in endometriosis. He stated that ectopic decidua on the pelvic peri-

toneum was first described by Walker³ in 1887 and the observation was soon confirmed by other workers. It is interesting to note that eleven of Weller's twelve references to the subject are in the German literature.

Ectopic decidual formation probably occurs more often than is generally appreciated. For example, Williams⁴ found ectopic decidual tissue in every fourth or fifth uterus removed at operation or autopsy at the end of pregnancy, an incidence of 20 to 25 per cent. Similarly, Hofbauer⁵ demonstrated ectopic decidua on the posterior aspect of the pregnant uterus in 15 of 23 specimens examined.

The ability of the tube to undergo decidual reaction was for many years a matter of controversy but it is generally recognized at the present time that such reactions do occur. Most authors agree that the tubal decidual reaction is slight in amount and patchy in location, approaching in no way the extensive decidual membrane formed in the uterus. The great majority of observations have been in connection with tubal pregnancy, particularly in reference to decidual formation at the implantation site. Williams⁴ stated that "occasionally, in uterine pregnancy, decidual cells may develop in the stroma of the tubal mucosa, but they never lead to the formation of a continuous membrane as in the uterus. Such observations are of extreme rarity but I have made them in several instances."

Kline⁶ studied 74 cases of extra-uterine pregnancy and presented evidence indicating that a decidual reaction of greater or less extent occurred constantly at the site of implantation. However, he observed a distant decidual reaction in the tube in only 3 of 51 cases examined. Kline thought that the decidua at the implantation site involuted rapidly with degeneration of the chorionic villi, whereas distant decidua in the tubes, uterus, or elsewhere sometimes persisted after degeneration of the trophoblast and complete involution of the local decidua. The observations in the present series of cases would seem to confirm this statement.

According to Frankel and Schenck,⁷ decidual tissue was described in tubal pregnancy by Webster⁸ in 1897. These authors further stated that Osiakina and Schmatok,⁹ in 1933, found a decidual reaction in the tube in 21 per cent of a series of 21 tubal pregnancies independent of the localization of the implanted ovum.

Geist and Matus¹⁰ stated: "It also seems likely that decidua is much more common in the tube than has been hitherto believed and that study of sufficient material may demonstrate this." These workers found decidual tissue most often in the connective tissue septa of the villi and believed that it "persisted for a much longer period of time

[than uterine decidua] because the involutionary process in the tube at this depth is decidedly slower than in the uterus."

Although a complete historical review was not possible, no report similar to the present one was found. In general there appears to have been surprisingly little written on the subject of ectopic decidual reactions.

DISCUSSION

The demonstration of decidual tissue in 12 per cent of 144 tubes removed post-partum is of theoretic importance in the etiology of tubal pregnancy. It is generally taught that the most important etiologic factors are mechanical ones. This study indicates that unusual receptivity of the tubal mucosa to the fertilized ovum may play a more important rôle than is generally believed. This idea has been advanced particularly by Frankel and Schenck,⁷ who believe that ectopic pregnancy results from implantation of the fertilized ovum on a receptive nidus of aberrant endometrium in the tubal mucosa or elsewhere. They believe that such a nidus of uterine mucosa is a prerequisite for the implantation and development of the ovum in a site remote from the uterus. This theory has much to recommend it but, as yet, proof is lacking. In not a single instance in the present study was aberrant uterine mucosa found. The extensive formation of decidua encountered in at least 3 of our cases suggests, nevertheless, that receptivity of the tubal mucosa may be an important factor in the causation of tubal gestation, entirely apart from the presence of actual uterine mucosa.

This statement is of particular significance in view of the high incidence of ectopic gestations in Hawaii. Schattenburg¹¹ has recently investigated this question. He found that during the 5-year period from 1936 to 1940 inclusive there were 4,964 deliveries and 75 ectopic pregnancies at The Queen's Hospital. The total number of tubal pregnancies thus comprised 1.5 per cent of the deliveries, a much higher figure than the ones usually given. For example, Schumann¹² stated that 1 in every 303 pregnancies is extra-uterine, an incidence of only 0.303 per cent. Salpingitis, considered to be one of the chief predisposing factors in the causation of ectopic gestation, is relatively infrequent in Hawaii. Perhaps this is a reflection of the exceptionally low venereal disease rate which exists here.¹³ May not the high incidence of ectopic gestations in Hawaii result from unusual hormonal "responsiveness" of the tubal mucosa, perhaps due to local climatic or racial factors? The present report suggests this since 11 of the 17 patients evincing a tubal decidual reaction were Hawaiians or

part Hawaiians and there were only 3 Caucasians in the remaining 6.

Be that as it may, a knowledge of the various stages in the involution of ectopic decidual cells is of practical importance to the pathologist. Interpretation is difficult when the peculiar ring forms associated with this process are observed for the first time. The degenerative process, when present, was identical in both the mucosal and serosal decidua and we have likewise found it in 3 of 5 cases of endometriosis associated with pregnancy. It may also be observed in uterine decidua, although the changes are not so well defined since the factor of inflammation is almost invariably present. Hofbauer,⁵ in his excellent paper published in 1929, noted the variation in size and shape of ectopic decidual cells and observed that vacuoles in the cytoplasm were common. We feel sure that cytoplasmic vacuolization is an initial stage in decidual cell involution.

The decidual cells arise from mesenchymal cells in the stroma of the folds and from similar cells beneath the serosal mesothelium. This process was described by Hofbauer⁵ who believed that such multipotent mesenchymal cells could give rise, not only to decidual cells, but also to unstriated muscle fibers. There was not the slightest suggestion in any of our cases that the decidual cells were derived from the tubal epithelium; the latter was always flattened by pressure. It is equally unlikely that the cells of the serosal mesothelium ever give rise to decidua. The serosal mesothelium could usually be recognized as a distinct although greatly flattened layer over the patches of decidua; elsewhere it was often hyperplastic and appeared cuboidal, even columnar, in cross section.

SUMMARY

Decidual tissue was demonstrated in one or both tubal segments from 17 of 144 post-partum sterilizations, an incidence of 12 per cent.

In 9 cases the mucosal folds were involved, in 5 there were patches of decidua beneath the serosa, and in 3 decidua was present in both locations.

The decidual tissue varied in amount from one or two cells in the tip of a mucosal fold in 2 cases to masses of cells in all of the mucosal folds in 3. Serial sections in the latter 3 cases showed the decidual tissue to extend longitudinally the entire length of the tubal segment.

In 8 of the 17 cases decidual cell involution of varying degree was present. This process is characterized by vacuolization of the cytoplasm and flattening and peripheral displacement of the nucleus. The cytoplasm then disappears entirely, resulting in peculiar irregular ring forms somewhat resembling the signet ring cells of mucoid ad-

enocarcinoma. The final stage is represented by faintly staining, small, irregular shadow or ghost forms.

The decidual cells arise from mesenchymal cells in the stroma of the folds and from similar cells beneath the serosa. The tubal epithelium over the areas of decidual tissue formation is greatly flattened by pressure; elsewhere it is normal. The serosal mesothelium covering the patches of serosal decidua is similarly flattened; in other locations it is often hyperplastic, appearing cuboidal or even columnar in nature.

CONCLUSIONS

1. This study indicates that decidual reactions in fallopian tubes may occur more often than is generally believed.
2. The fact that 67 per cent of the patients showing a tubal decidual reaction were Hawaiians or part Hawaiians, together with our inability to find a similar report in the literature, suggests, however, that the phenomenon may be a purely local one, perhaps dependent upon racial or climatic factors.
3. It is further suggested that it may have an etiologic bearing upon the high incidence of ectopic gestation in Hawaii.

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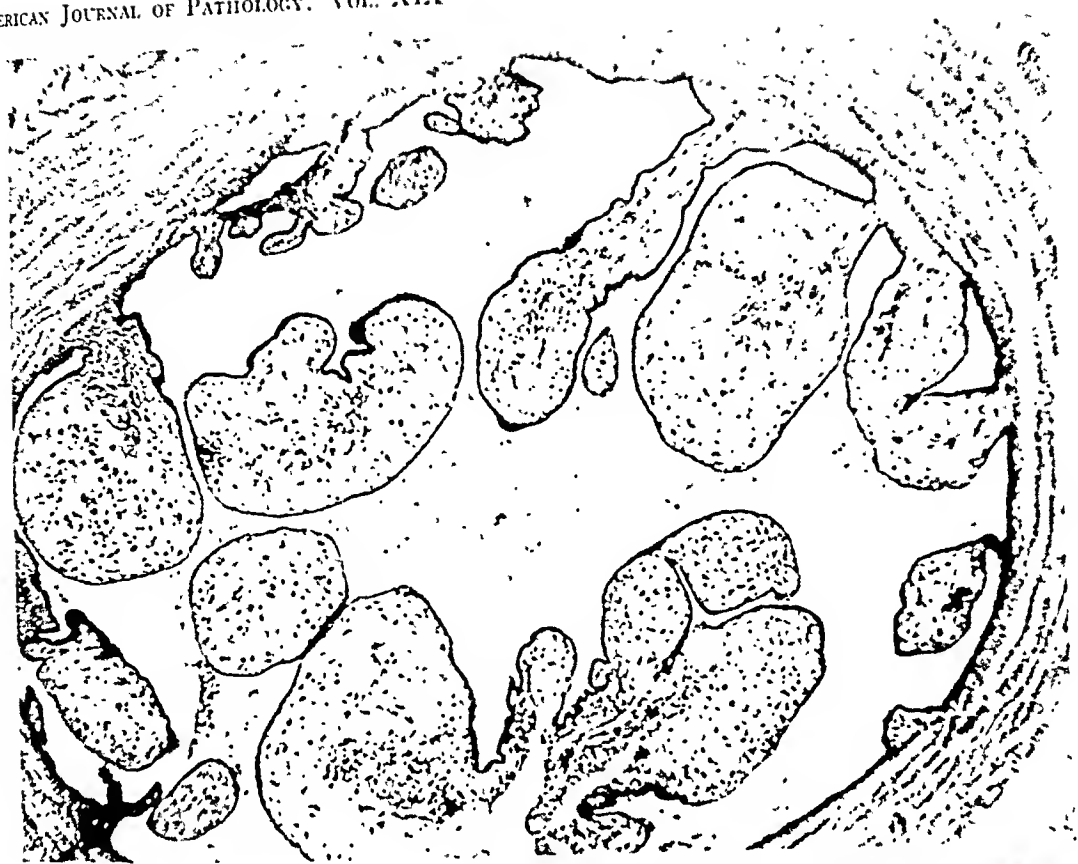
[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 126

- FIG. 1. Case 1. Every mucosal fold is distended by decidual cells. Advanced involution is present, leaving most of the folds partially cystic. Hematoxylin and eosin stain. $\times 40$.
- FIG. 2. Case 1. A distended mucosal fold attached to the wall by a relatively narrow pedicle. The tubal epithelium is flattened by compression. Extensive involution is present, leaving the fold partially cystic. The remaining decidual cells are in various stages of degeneration and vacuolated cells and ring forms are evident. Hematoxylin and eosin stain. $\times 130$.
- FIG. 3. Case 5. Two structurally intact decidual cells are present in the tip of a mucosal fold. The covering epithelium is flattened. Hematoxylin and eosin stain. $\times 300$.

1



2



3



PLATE 127

- FIG. 4. Case 8. A mucosal fold distended by a mass of structurally well preserved decidual cells. The cells vary somewhat in size and shape and the covering tubal epithelium is flattened. Hematoxylin and eosin stain. $\times 300$.
- FIG. 5. Case 16. A group of decidual cells in a peritoneal evagination near the mesosalpinx. Here are seen vacuolated cells, ring forms and an intact but greatly flattened covering layer of serosal mesothelium. The structure below is cuboidal mesothelium which has become detached from the underlying tissue. Hematoxylin and eosin stain. $\times 300$.
- FIG. 6. Case 1. Involuting decidual cells. Most of them exist as peculiar, somewhat irregular ring forms. Hematoxylin and eosin stain. $\times 300$.
- FIG. 7. Case 12. The serosal mesothelium is cuboidal in appearance, the cells rounded due to cytoplasmic increase, and the nuclei are located centrally. No decidual cells are present. Hematoxylin and eosin stain. $\times 300$.



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